STEREOSELECTIVE ASPECTS OF HALLUCINOGENIC DRUG ACTION AND DRUG DISCRIMINATION STUDIES OF ENTACTOGENS

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To my parents with warm appreciation for their love and support.

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ABBREVIATIONS AND NOMENCLATURE

AMP	amphetamine
BL-3912	1-(2,5-dimethoxyphenyl)-2-aminobutane (BL-3912A = *R*-(-)-isomer)
95% C.I.	95% Confidence Interval
DD	Drug discrimination
DOM	1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane
DS	Discriminative stimulus
EDA	3,4-ethylidenedioxyamphetamine
ED_{50}	Median effective dose - a measure of potency for test drugs: the dose required to produce drug lever selections in half the responding rats.
HRMS	high resolution mass spectrometry
IDA	isopropylidenedioxyamphetamine
IR	infrared spectroscopy
Log P	logarithm of the octanol/water partition coefficient
MA	methamphetamine, methamp
MBDB	1-(1,3-benzodioxole-5-yl)-2-butanamine, or 1-(3,4-methylenedioxyphenyl)-2-methylaminobutane
MDA	3,4-methylenedioxyamphetamine
MDE	3,4-methylenedioxy-*N*-ethyl-amphetamine
MDMA	3,4-methylenedioxymethamphetamine
MDAI	2-amino-methylenedioxyindan
MDAT	2-amino-methylenedioxytetralin
m/e	mass divided by charge

mg/kg	milligrams per kilogram
5-MeO-DMT	5-methoxy-*N*,*N*-dimethyltryptamine
mL	milliliter
MPB	2-methylamino-1-phenylbutane
μM/kg	micromoles per kilogram (μmol/kg)
NMR	nuclear magnetic resonance
N-OH-MDA	*N*-hydroxy-3,4-methylenedioxamphetamine
pK_a	negative logarithm of the acid dissociation constant
SAR	Structure-activity relationship, QSAR = Quantitative SAR
LSD	*d*-lysergic acid diethylamide

ABSTRACT

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Stereochemical aspects of the structure-activity relationships of hallucinogenic drugs were explored through the asymmetric syntheses and pharmacological evaluations of two sets of analogues. The two lever drug discrimination (DD) paradigm was utilized with LSD (0.08 mg/kg) as the training drug. Four stereoisomeric derivatives of LSD were prepared in which the diethyl amide moiety was replaced with a 2-chloro-1-methylpropyl amide group. All of these analogues completely substituted for LSD with a 22-fold potency range and one of these novel derivatives exhibited a potency greater than LSD itself. The results are discussed in terms of the role of the amide portion of hallucinogenic ergolines in the drug-receptor interaction.

Steric effects on the behavioral activity of phenethylamine type hallucinogens were investigated by comparing the DOM analogues 1-[2,5-dimethoxy-4-(2-methylpropyl)phenyl]-2-aminopropane, and 1-[2,5-dimethoxy-4-(2-butyl)phenyl]-2-aminopropane. The effect of stereochemistry at the 4-position of these analogues was also studied. An asymmetric hydroboration procedure was employed in the preparation of the *R* and *S* 4-secbutyl analogues of DOM. The results suggest that steric bulk at the 4-position attenuates activity to a greater degree when it occurs close to the aromatic ring. A modest difference in the activities of the optically active derivatives was also observed and the results are discussed in terms of the possible role of charge-transfer complexation between hallucinogenic phenethylamines and the receptor.

Finally, a series of drug discrimination experiments was performed in order to test the hypothesis that 3,4-methylenedioxymethamphetamine (MDMA)-like compounds, termed *entactogens*, exert a novel type of psychopharmacology. Thus, the racemic mixtures and optical isomers of MDMA, the primary amine derivative, MDA, as well as MBDB, the α-ethyl homologue of MDMA, were synthesized and compared with respect to discriminative stimulus properties. Groups of rats were trained to discriminate saline from (±)-MDMA (1.75 mg/kg), (+)-MBDB (1.75 mg/kg), LSD (0.08 mg/kg), or (+)-amphetamine (1.0 mg/kg) and substitution tests, as well as a limited number of antagonist tests, were performed. The results clearly indicate that MDA, MDMA and MBDB share a common component of behavioral activity which is distinct from both hallucino-gen-like and stimulant-like effects. The structure-activity relationships of entactogens were also explored and found to be consistent with the concept that entactogens represent a novel class of drugs. In addition, since MBDB and the rigid analogue, 5,6-MDAI, lack the stimulant-like effects of MDMA, these drugs will be useful tools in future studies.

CHAPTER 1 - INTRODUCTION

The significance of the types of drugs which are the subject of this research, hallucinogens and entactogens, can be viewed from several perspectives. In the work described here, the study of these compounds has been approached from the point of view of developing neurochemical tools that can ultimately help to elucidate mechanistic aspects of brain function. Snyder (1986) has noted that the research of the past decade has proceeded at a furious pace which may, in the near future, lead to unparalleled advances in our understanding of the brain and in our ablility to put this understanding to therapeutic use. The goal of this work is to contribute efforts toward that end.

Organizational Outline

The common threads of the three chapters that follow are first, the focus on structure-activity relationships (SARs), and second, the use of the drug discrimination (DD) paradigm to assay *in vivo* activity. Chapters 2 and 3 describe the synthesis and evaluation of novel analogues of the prototypical hallucinogens LSD and DOM (STP), respectively. The project described in Chapter 2 was directed toward understanding the apparent necessity of LSD's diethyl amide for optimum potency. In Chapter 3 the influence of the position and stereochemistry of a methyl branch in the *para*-alkyl substituent of hallucinogenic "amphetamines" on activity is considered. The novel psychopharmacology of certain methylenedioxy-substituted phenethylamine derivatives is the subject of the final chapter. Experiments are described which test the hypothesis that these agents, referred to as entactogens, represent a new class of drugs with effects that can be distinguished from those of hallucinogens and stimulants.

This introductory chapter will begin with a general discussion of the definitions and background of hallucinogens and entactogens. Some general considerations of SARs and the importance of stereochemistry in drug action will then be reviewed. Following that will be a discussion of the problems that have been encountered while trying to evaluate hallucinogenic activity and why the drug discrimination paradigm is particularly useful in this regard. Since the mechanisms of action of these drugs seem to involve serotonergic neurons, the chapter concludes with a brief discussion of this neurotransmitter system and current concepts of hallucinogen and entactogen drug action.

Within each chapter, the results of the DD testing are included in the form of summary tables accompanied by references to the original data. The latter, along with the details of the DD methodology, have been included in the appendices. In Chapters 2 and 3, the experimental sections for the synthetic chemistry procedures are presented last, in order to facilitate reading.

Definitions and Background

Hallucinogens, found naturally in numerous plant species, represent one of the oldest known types of psychoactive substances and have a rich folkloric history. Entactogens, by contrast, have only very recently been introduced as a distinct drug class (Nichols et al., 1986a) with no known natural sources but with perhaps a great deal of potential as tools for psychiatry. Since entactogens can be viewed as originating from hallucinogens, the latter will be discussed first.

Hallucinogens

Although mankind has known about and used hallucinogenic substances for centuries (Schultes, 1978), hallucinogens have no accepted medical use today despite the general recognition that, as Hollister (1978) has noted, few drugs have had more scientific or social impact. The difficulty in defining precisely what hallucinogenic activity means is reflected in the vast number of synonyms that have evolved. Shulgin (1978) has listed no less than twenty different names that have been proposed, and commented that if there is confusion in choosing a term to describe this class of drugs, then there is chaos in agreeing upon a description of their effects. The most often used synonyms are "psychotomimetic", implying the production of a "model psychosis", and "psychedelic" meaning "mind manifesting."

The term hallucinogen is still used most frequently in the scientific literature but not without controversy. It can be defined as describing a substance which produces perceptions, in any of the senses, that take place in the absence of environmental stimuli. Snyder (1986), among others, has argued against the use of this term since these drugs do not typically produce hallucinations but cause existing perceptions to be distorted. Siegel (1984), has noted that although the word hallucination was first used in the English language in 1572 by Lavater, and described "ghostes and spirites walking by nyght," the term was originally derived from the Latin "alucinatio," meaning a wandering in mind, idle talk, or prating.

These difficulties in definition may reflect the limitations of our language, which should not be viewed as a trivial matter. Indeed, Walsh (1980) has asserted that English is poorly equipped to deal with precise descriptions and analysis of consciousness. And "since 'we dissect nature along lines laid down by our native languages', which form the basis for our social construction of reality, our linguistic limitations may limit our understanding and development in the areas of consciousness disciplines" (Walsh 1980).

Having mentioned this controversy, excess confusion can be avoided by settling on a practical definition. Jaffe (1985), in the 7th edition of Goodman and Gilman's The Pharmacological Basis of Therapeutics, states that, although these drugs can produce pathological effects implied in the terms hallucinogenic, psychotomimetic, and psychotogenic, "the features that distinguish the psychedelic agents from other classes of drugs is their capacity to reliably induce or compel states of altered perception, thought, and feeling that are not (or cannot be) experienced otherwise except in dreams or at times of religious exaltation." The comparison of the effects of hallucinogenic agents with dreaming has also been made by Albert Hofmann (1959), the Swiss chemist who first synthesized and discovered the effects of LSD, in describing the experience:

> The psychotomimetics produce profound and astute changes in the sphere of experience, in the perception of reality, changes even in space and time and the consciousness of self. Phenomena of depersonalization may also occur. Retaining full consciousness, the subject experiences a kind of dream world. Objects and colours, which generally become more brilliant, lose their symbolic character, they stand detached and assume an increased significance, having, as it were, their own more intense existence.

The range of experiences afforded by hallucinogenic substances is described by Masters and Houston (1967) and the interested reader may refer to that book, among many others, for further information.

For most of man's history, the use of hallucinogenic substances was almost exclusively in the context of religious activities involving mystical states (Jacobs, 1984). In modern times, interest in the effects of these compounds was stimulated by the discovery, in 1943, of the temporary but powerful psychological changes caused by remarkably low doses of LSD. When Sandoz first offered to supply LSD, marketed under the trade name Delysid, to select researchers in 1947, the accompanying literature suggested two possible uses (Stevens, 1987):

> Analytical: To elicit release of repressed material and provide mental relaxation, particularly in anxiety states, and obsessional neuroses.
> Experimental: By taking Delysid himself, the psychiatrist is able to gain insight into the world of ideas and sensations of mental patients. Delysid also can be used to induct model psychoses of short duration in normal subjects, thus facilitating studies on the pathogenesis of mental illness.

A large number of clinical research projects were conducted with LSD during the ten years that followed, but these tapered off in the next decade. At the time of this intense study, knowledge of central nervous system (CNS) pharmacology was rudimentary, and the presence of amine neurotransmitters in the brain and their link to behavior had just been unveiled (Freedman, 1984).

News of these experiments made its way out of the laboratory, stimulating the interest of numerous artists, writers and others who were fascinated with the effects of hallucinogens. When LSD entered the "beat scene" in the late 1950's it quickly became the method of choice for achieving "that ancient heavenly connection" (Stevens, 1987).

Many young people viewed the psychedelic experience as valuable both for adapting to and producing social change, which generally precipitated reactions of resistance and fear from people who were concerned about the negative effects that drug use and abuse seemed to be having on individuals and on society in general. Sensationalized accounts of LSD use became abundant in the media and laws which made the possession of hallucinogens a criminal offense were enacted. Clinical research ended shortly thereafter.

In summing up the current state of affairs regarding clinical LSD use, the following comments of Grinspoon and Bakalar (1983) are worth noting: "It is good that these drugs are no longer fashionable, but unfortunate that using them for serious purposes has also been made almost impossible" due to the entanglement of this issue with "the general problem of drug abuse and the special cultural conflict of the late 1960's and early 1970's."

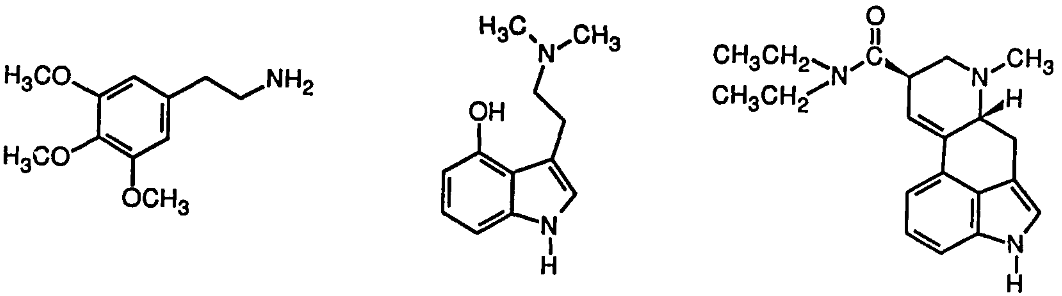
However, many interesting clinical studies of LSD were published, including a variety of experimental psychiatric applications (see review by Pahnke, et al., 1970). It has been suggested that LSD can be usefully employed in the treatment of certain patients with specific disorders, such as chronic alcoholics and sexual deviates (see references in Valzelli, 1973). For example, in a case report described by Brandrup and Vanggaard (1977) the emergence of childhood memories and the simultaneous disappearance of symptoms of a severe compulsive neurosis was observed in a patient given LSD in psychotherapy. One psychiatrist, Stanislav Grof, employed hallucinogens in clinical treatments and, as a result, developed new theories regarding the etiology and treatment of certain emotional illnesses (Grof, 1978). Based on the results of clinical research, Pahnke et al. (1970) offered the following comments:

> Our hope is that the current public concern over abuse can give way to a resurgence of careful and informed research including specialized training and experience for those interested in this area. Our past and present research continues to intrigue us with the promise of psychedelic drugs, but they are obviously powerful tools that need to be used and handled wisely. Hopefully, it is not too late for them to be harnessed for medical and research use in a variety of ways in the future.

Fourteen years later, Strassman (1984) reached similar conclusions in expressing the need, after a decade of inactivity in this area, to begin reinstituting psychedelic research in humans. Despite widespread fears of possible harmful physical effects, numerous investigations have failed to provide conclusive evidence of physiological damage produced by LSD, and under proper conditions the incidence of adverse psychological reactions is low (Pahnke et al., 1970; Strassman, 1984).

Therefore, the significance of medicinal chemistry studies of hallucinogens may be viewed in terms of their demonstrated and potential value as therapeutic tools, in addition to their value in basic research. It is generally recognized that hallucinogens are very useful as pharmacological probes of basic neurophysiological processes, and as tools by which to understand mood and perceptual disturbances (Heym and Jacobs, 1988). "By studying the actions of psychedelic drugs", notes Snyder (1986), "scientists hope to learn about the brain processes responsible for the way that we perceive both ourselves and the relationship with the world outside ourselves."

Chemically, there are three types of compounds that comprise this class of drugs. The first type is based on the relatively simple ß-phenethylamine nucleus, the prototype of which is mescaline. The second type is based on the tryptamine nucleus, where the prototype is psilocin, while the third type is based on the chemically more complex ergoline structure (LSD). These prototypes are shown in Figure 1.



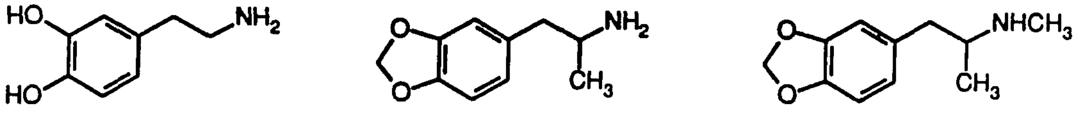
Mescaline Psilocin LSD

Figure 1. Examples of the three chemical types of hallucinogens.

While the potencies of these prototypical hallucinogens differ dramatically, the clinical syndromes which they produce are virtually identical to one another (Hollister, 1986). However, meaningful SAR comparisons generally require the separate study of phenylalkylamines and indolealkylamines. The lack of a comprehensive hallucinogen SAR may be related to metabolic and distributional differences between the two types of aromatic ring systems (Nichols and Glennon, 1984).

Entactogens

In contrast to the common activity of these three types of hallucinogens, a small series of compounds related chemically to the hallucinogenic phenethylamines seems to produce distinctly different effects. Methylenedioxyamphetamine (MDA), and its *N*-methyl derivative, methylenedioxymethamphetamine (MDMA) are two members of this series. Both are chemically similar to the neurotransmitter dopamine, as illustrated in Figure 2.



Dopamine **MDA** **MDMA**

Figure 2. Molecular structures of dopamine, MDA and MDMA

As reflected in their names, and by virtue of their α-methyl groups, MDA and MDMA are commonly considered derivatives of amphetamine (alpha-methylphenethylamine). However, evidence will be presented in Chapter 4 which suggests that the methylenedioxy substituent influences their biological activity in a way that makes them distinct from stimulants such as amphetamine.

Both MDA and MDMA were first prepared in the early part of this century (Merck, 1914). Alles (1957) reported on the unusual effects of MDA, based on self-experimentation. In the 1960s, MDA became a popular recreational drug, producing "an almost overwhelming desire to be with and talk to other people" and an "increased need for interpersonal communication" (Jackson and Reed, 1970). Clinical investigations of the drug (Naranjo et al, 1967; Yensen et al., 1976) evaluated its usefulness as an adjunct to psychotherapy. The effects of 100-150 mg of MDA have been described by Weil and Rosen (1981):

> ...it produces a feeling of physical and mental well-being. In the drug subculture, MDA is known as the "love drug" because it's supposed to inspire loving feelings in groups of people. Unlike mescaline, it rarely changes visual perceptions. Although it is a strong stimulant, chemically related to amphetamine, people who take it say it calms them and promotes relaxation.

In a clinical report on the subjective effects of MDA (Turek et al., 1974), comparisons with a similar study using LSD indicated that:

> ...LSD is more likely than MDA to produce unitive experiences involving a sense of ego loss and to provide access to dimensions of awareness that are beyond the usual conceptions of time and space. The LSD experience is also more difficult to describe in logical, rational terms.
>
> MDA-produced changes experienced by the majority of subjects were reflected in the following items in the questionnaire: feelings of peace and tranquility, feelings of tenderness and gentleness, increase in the beauty and significance of music, feelings of emotional closeness with the companion, increased awareness of the importance of interpersonal relationships...

These descriptions suggest that MDA may be inducing behavioral effects through mechanisms different from those of stimulants and hallucinogens. More recently, anecdotal reports similarly suggested that MDMA's psychopharmacology was not readily categorized within existing classes of psychoactive agents. The drug reportedly "does not produce the high of marijuana, the rush of cocaine or amphetamines (speed) or the hallucinations of LSD" (Touflexis, 1985). The idea that MDA might represent a unique drug class was first proposed on the basis of data from animal studies by Shannon (1980).

Additional data seemed to support the concept that the primary activity of MDA is novel, but is complicated by hallucinogen-like and stimulant-like activities. Furthermore, although the primary activity of MDMA is similar to that of MDA devoid of hallucinogen-like effects, some stimulant-like effects were still observed (Nichols, 1986b). The concept of novel activity gained support from studies with the α-ethyl homologue of MDMA, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB, Figure 3).

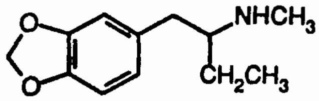


Figure 3. The molecular stucture of MBDB.

By replacing the α-methyl, a distinguishing characteristic of amphetamine derivatives, with an α-ethyl, the stimulant-like activities of MDA and MDMA were abolished. In addition, since the combination of the α-ethyl and *N*-methyl were anticipated to provide synergistic attenuation of hallucinogen-like activity, MBDB represents a drug with a much simplified pharmacology (Nichols et al., 1986; Oberlender and Nichols, 1988). As described in Chapter 4, the data suggest that the primary effects of MBDB, MDA, and MDMA are similar.

A new psychoactive drug class was therefore proposed for compounds with this type of activity. The term entactogen was introduced as a name for this class and MBDB was suggested as the prototype (Nichols et al., 1986b). The word entactogen was described as being "derived from the Greek roots 'en' for within or inside and 'gen' to produce or originate and the Latin root 'tactus' for touch. Hence, the connotation of this word is that of producing a 'touching within'" and reflects the drug's ability to facilitate communication and introspective states" (Nichols et al., 1986b).

Over the past four years, there has been a considerable surge of interest and research associated with these compounds. A major factor responsible for the increased attention entactogens have received was concern over an apparent dramatic increase in the recreational use of MDMA. Given the street name "Ecstasy," the drug was the focus of widespread coverage in the news media (Adler et al., 1985; Touflexis, 1985; Leavy, 1985; Gertz, 1985). The controversy involved the Drug Enforcement Administration's (DEA) decision to classify it as a Schedule I controlled substance. This category is used for drugs which have high abuse potential, no accepted medical use, and lack accepted safety for use under medical supervision.

This otherwise routine story attracted additional attention when the DEA's decision was challenged by a group of psychotherapists who had been using MDMA as an experimental tool by which to facilitate the therapeutic process. Although controlled scientific studies of the drug's therapeutic efficacy were not conducted, significant benefits were claimed by psychiatrists who were studying MDMA-assisted psychotherapy in their practices (e.g. Greer and Tolbert, 1986). These benefits include the ability of the drug-induced state to help people to get in touch with feelings which are ordinarily not available to them, and to recall experiences from the past, overcome irrational fear, and improve self esteem (Adler et al., 1985).

Thus, interest in the potential medical use of MDMA had developed before its widespread recreational use began. Since the patent for MDMA had long since expired, the commercial interest necessary to fund preclinical studies required for FDA approval did not exist. Yet, prior to scheduling, an estimated 30 psychiatrists and a larger number of therapists were thought to be working with it (Adler et al., 1985). Meanwhile, the nonmedical use of MDMA escalated. Nationwide, an estimated 10,000 doses were distibuted in 1976, which increased to 30,000 per month in 1985 (Leavy, 1985). One study reported an estimated 39% of undergraduates at Stanford University had tried MDMA at least once (Peroutka, 1987).

Although MDMA was never produced commercially, at the same time, it was not explicitly banned. With its growing popularity came flagrantly open sales of a "legal high." Cases of abuse also began to surface and by the summer of 1985, DEA administrator John Lawn stated that MDMA had become a nationwide problem posing a serious health threat (Touflexis, 1985). An article in Newsweek included the assessment that "apparently, the nation is on the verge either of a tremendous breakthrough in consciousness or a lot more kids too strung out to come in from the rain" (Adler et al., 1985).

The therapists agreed that some controls were needed but attempted to pursuade the government to place MDMA into Schedule III rather than Schedule I. They argued that the development of tolerance seemed to limit the drug's abuse potential and that investigational medical use had already been accepted. As a result of this proposed change in scheduling, Harvard psychiatrist Dr. Lester Grinspoon noted that "the law would still have what it needs, but it wouldn't retard the kind of research that we need" (Touflexis, 1985).

In November of 1984, the DEA "referred the matter to the Agency's Administrative Law Judge, Francis L. Young, to conduct a hearing for the purpose of receiving factual evidence and expert opinion regarding the proposed scheduling of MDMA" (Mullen, 1984). Judge Young recommended placement of MDMA into Schedule III but the DEA disagreed and as of October, 1986, MDMA remained in Schedule I. Following this, an opinion on a Petition for Review was issued by the U.S. Court of Appeals in September, 1987. It remanded the ruling for further consideration, based on an erroneously applied interpretation of the law. The DEA's final ruling placed MDMA into Schedule I (Lawn, 1988).

Roberts (1986) has argued that the MDMA issue is not just a question of which schedule it should be assigned to or whether the drug has psychotherapeutic potential. In that MDMA and other state of consciousness "psychotechnologies" represent methods of exploring the human mind, Roberts suggests that the questions surrounding this issue deserve close attention. The prohibition of such methods, he argues, may severely limit our knowledge of human nature. Tart (1972) has also pointed out the waste of valuable learning that results from not developing the scientific study of altered states of consciousness.

Although any further discussion of such questions is beyond the scope of the present work, observations such as these highlight the need to understand the medicinal chemistry of MDMA and similar compounds. In addition, another facet of the MDMA issue has become increasingly prominent. Evidence has been accumulating from animal studies which indicates that high doses can cause neurotoxicity, in the form of specific degeneration of certain serotonergic nerve terminals (Barnes, 1988). Whether or not similar effects occur in humans, at behaviorally relevant doses, is as yet unknown. The reversibility of such an effect and the relationship between the mechanisms of neurotoxicity and behavioral activity are still not understood. It is clear, however, that clinical testing of MDMA itself will probably not be pursued in the forseeable future.

The goals of the research in this area can be broadly defined as follows. Experiments were directed primarily toward elucidating the SAR and mechanism of action of entactogens. Using rats trained with various agents, the DD paradigm was used to test the hypothesis that entactogens represent a unique drug class. An additional focus of this work involved the drug development aspect of medicinal chemistry. Nichols (1987) has discussed the apparent stagnation in the field of drug-assisted psychotherapy research and noted that the chemical tools available to psychiatrists are "woefully limited" compared to other specialties in medicine. In fact, it seems that very little has changed since Glaser (1971) noted that psychotherapy is an evolving method and there is no reason to think that it cannot evolve further. The eventual development of the therapeutic applications of entactogens may therefore catalyze significant advances in psychiatry.

Thus, using animal models, the search has begun for entactogens which can produce the characteristic effects of MDMA that psychiatrists might find valuable, without side effects. An ideal entactogen would also have minimal abuse potential and would be devoid of neurotoxic potential. The development of such an agent would then allow clinical testing for safety and efficacy as an adjunct to psychotherapy.

General Considerations of Structure-Activity Relationships

Over 100 years ago, Crum-Brown and Fraser (1869) suggested that biological activity must in some way be related to the structure of the bioactive agent. Since that time, SAR methodology has advanced significantly (Hansch, 1973; Portoghese, 1970; Pfeiffer and Jenney, 1967; Lehmann et al., 1976). The assessment of these relationships provides a powerful tool for developing improved medicinals and studying drug action.

The process of developing SARs involves the identification of a prototype drug or lead compound which elicits a characteristic effect, e.g. LSD or MDMA. One also needs to define a specific, measurable biological effect which can be quantitated. In the present work, DD methodology (see appendix A) was employed for that purpose. The next step involves the synthesis and evaluation of a congeneric series of compounds based on the lead molecule.

Speculation concerning the causes of the observed differences in activity can be derived from the results of such experiments and a knowledge of the physicochemical properties associated with the various structural features. Taking into account pharmacokinetic factors, the interaction between a drug and its receptor can be studied by observing how biologic potency is affected by each molecular modification. (Goldstein et al., 1974). Many studies have been designed over the years to attempt to define the functional topography of a receptor indirectly from the structure of the drugs with which it interacts (e.g. Nichols, 1985). Thus, drugs with higher affinity for a particular receptor are presumed to be more structurally complementary to the site. This serves as the basis for much of the work described in the subsequent chapters.

The forces that bind the two are essentially those by which all organic molecules interact (Taylor and Kennewell, 1981). For example, all of the drugs that will be discussed in the subsequent chapters contain a basic nitrogen atom which is appreciably ionized at physiological pH. This positively charged part of the molecule is thus capable of interacting with anionic groups, such as ionized carboxyl and phosphate groups of the biological target site's macromolecular structure (Smith and Williams, 1983). Other chemical forces thought to be important in drug-receptor interactions include ion-dipole and dipole-dipole interactions, hydrogen bonding, charge-transfer complexation, hydrophobic binding and van der Waals' forces (Taylor and Kennewell, 1981).

The mechanisms of action for various drugs can also be studied using SARs. This can be done, for example, by attempting to correlate the *in vivo* potencies of compounds in a congeneric series, with the potencies in producing an *in vitro* or biochemical activity. A high correlation provides evidence of a relationship between the two. In addition, the compounds that are synthesized in SAR studies may prove to be useful as pharmacological tools in other research. To a large extent, the identification and study of various neurotransmitter receptor types and subtypes is dependent on the availability of agents which interact selectively with these biological targets. New compounds that are useful in receptor binding studies can be extremely valuable for increasing our understanding of neurochemical events and the behaviors associated with them.

SAR results are also useful in the design of new and better drugs. The growth in the science of drug design has been accompanied by a shift from qualitative to quantitative aspects. This involves the expression of SARs in terms of a mathematical equation. On one side of the equation is a variable representing some relevant aspect of a drug's biological activity. The other side contains variables related to various properties of the molecular structure. These are typically functions relating to electronic, steric and hydrophobic properties. Unfortunately, quantitative structure-activity relationship (QSAR) studies with hallucinogens have not been very fruitful (Gupta et al., 1983).

Stereochemistry in Drug Action

Much of the research on entactogens and hallucinogens which will be described here involves stereochemical apsects of drug action. Stereoisomers, in general, are molecules composed of the same constitutent atoms, having the same structural formula, but which differ with respect to the spatial arrangement of certain atoms or groups of atoms. The term enantiomer is used to describe stereoisomers that are mirror images of each other, while diastereomers are not mirror images.

When the biological activities of stereoisomers are compared, it is sometimes observed that only one member of each pair is active (stereospecificity), or more frequently, that both members exhibit the same type of activity but to a different degree (stereoselectivity), but rarely that both members are equally active (Lehmann, et al., 1976). Stereoselectivity in drug action is related to the differentiation of asymmetric molecules by asymmetric environments (Alworth, 1972).

When enantiomers are in chiral environments, such as when a drug approaches a receptor, the chiral "host" environment tends to interact selectively with the "guest" enantiomer with which it is more chemically complementary. "This can be visualised as a key and lock principle," as Ariëns et al. (1988) point out, "not as something static but as a dynamic process of mutual adaptation, an 'embracement' between substrate and enzyme, or messenger molecule and receptor."

The relative potencies of enantiomers has been explained on the basis of a "three-point attachment" model originally proposed by Easson and Steadman (1933) and later modified (see references in Ruffolo, 1983). It postulated that the adrenergic receptor (later extended to the four known subtypes of this receptor) interacted with its natural ligand, norepinephrine (NE), at three dissymmetrically disposed sites on its surface.

As illustrated in Figure 4 (from Ruffolo, 1983), these sites were proposed to interact with various critical parts of NE: A, with the basic nitrogen; H, with the benzylic hydroxyl group; and P, with the phenyl group.

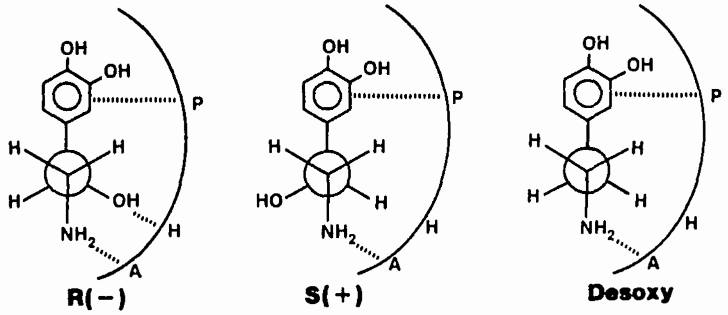


Figure 4. The Easson-Steadman three-point attachment model.

The natural *R*-(-)-isomer of NE (but not *S*-(+)-NE or dopamine, the ß-desoxy derivative) could adopt a conformation in which all three groups could interact with these sites. The latter two were oberved to have potency equal to each other but less than the natural isomer. Presumably, this was due to the difference between a two point and three point attachment.

In addition to pharmacodynamic differences resulting from binding interactions at the active site, enantiomers might also differ with respect to their pharmacokinetics (Drayer, 1988). This term refers to factors associated with absorption, distribution, metabolism, and elimination. The net result is that one isomer, the eutomer, is more potent in eliciting a certain activity than the other, the distomer. The degree of stereoselectivity is reflected in the eudismic ratio, the eutomer potency/the distomer potency (Ariëns, 1984).

Several characteristics of stereoselectivity have been elucidated. For example, the magnitude of the eudismic ratio depends on the location of the chiral center in the drug molecule in relation to the part of the molecule which interacts with the receptor. When the chiral center is close to the points of interaction it can exert a greater effect on binding and the eudismic ratio tends to be high (Lien et al., 1976). In such cases, a generalization known as Pfeiffer's rule becomes applicable: the lower the effective dose of a racemic drug, the greater the difference in the potency of the optical isomers (Pfeiffer, 1956).

Stereoselectivity is intricately related to the mechanisms of action of drugs and may therefore contibute insight into the molecular aspects of drug action (Ariëns, 1987). For compounds with multiple actions, the eudismic ratios can be calculated for each individual activity in order to determine whether the effects are based on a common mechanism, in which case the ratios will be comparable. In general, drugs which act through a common receptor can be expected to exhibit similar stereoselectivity, but the reverse is not necessarily true. Drugs which exhibit similar stereoselectivity may, of course, exert effects through unrelated mechanisms. Therefore, in considering stereospecificity in relation to the mechanism of drug action, the information should always be analyzed together with results from a variety of approaches before valid conclusions can be reached. Stereoselectivity is an important concept in many SAR studies. For example, hallucinogenic phenylisopropylamines have been found to exert greater activity when the isopropylamine side chain has the *R* absolute configuration (Dyer et al., 1973). However, care must be taken to avoid misinterpretations (Ariens, 1984; Oberlender et al., 1987).

Problems With Evaluations of Hallucinogenic Activity

As reflected in the controversy surrounding the various names that have been applied to the hallucinogen drug class, these compounds seem to exert effects that are often difficult to define, let alone to quantitate. This presents a critical problem in developing useful potency series for SAR evaluations (Nichols and Glennon, 1984), since the major limiting factor of these studies is the relevance and reliability of the pharmacological assay. Ideally, because these compounds affect higher level brain functions, one would like to have qualitative and quantitative data obtained from controlled clinical studies.

The most extensive data base of activity in humans has been developed by Dr. A.T. Shulgin. At a time when no other laboratories published in this area, Shulgin and co-workers have described human activity for approximately 200 psychoactive compounds (Shulgin et al., 1986). Without the data generated and published by this group, there would be no means by which to validate potentially useful animal models. White (1986) recently commented that "until more detailed, controlled and anecdotal clinical findings are forthcoming (which is unlikely given current constraints), we must rely on animal models." With that realization, attention has been focused on developing a useful assay.

Behavioral pharmacologists have employed a vast array of disparate techniques and animal species to catalogue the effects and to study the underlying mechanisms of hallucinogens (White, 1986). Nichols and Glennon (1984) have referred to the variety of paradigms that have been used over the last decade as illustrative of the long and frustrating search for valid animal models to correlate with clinical findings. These include, but are by no means limited to disruption of conditioned avoidance response in rats, mouse head twitch, rabbit hyperthermia, cat rage response, cat limb flick, mouse ear scratch, flexor and stepping reflex in chronic spinal dog, serotonin syndrome in rats, and two-lever drug discrimination in rats (Nichols and Glennon, 1984).

Description and Advantages of Drug Discrimination

The drug discrimination (DD) paradigm has developed into a powerful technique, valuable to medicinal chemists interested in SARs of centrally active compounds. The drug "states" produced by relatively low doses of hallucinogens, as well as a large and increasing number of other types of drugs, can be studied in a qualitative and quantitative manner, in terms of their discriminative stimulus properties (Appel et al., 1982; Overton, 1984). The particular usefulness of DD in the analysis of hallucinogenic drug actions has been attributed to the variability in the induced effects which makes analysis by less complex procedures too difficult (Cunningham and Appel, 1988).

In the commonly used two lever paradigm, the animal is presented with a left and right lever and must press the correct one in order to obtain a reward. Discrimination training teaches the animal, through a process called differential reinforcement, to respond on one lever after being given the training drug, and the other lever after being given saline. The choice of levers (left *vs.* right) is critically dependent on the subject's perceived state. Thus, the drug state serves as a stimulus, an interoceptive cue that provokes a response. The subject is required to discriminate the drug stimulus in order to respond by selecting the correct lever, hence, the term "drug discrimination." In addition to learning to discriminate between a drug *vs* non-drug (saline) state, the states produced by one drug *vs.* a different drug (White, 1986), or even two different doses of the same drug (Colpaert and Janssen, 1986) can be discriminated.

After the animal acquires the discrimination, test sessions are interspersed between training sessions. When the animal is presented with a new compound, such as a structural analogue of the training drug, he will respond on the drug-appropriate lever if that new compound exerts a pharmacological action similar to the training drug at the dose received (stimulus generalization). If the test drug is inactive, he will respond on the saline lever. If the test drug is active, but does not share a common mechanism of action with the training drug, the third state hypothesis (Winter, 1978) usually holds, and the animal will choose the saline lever. By testing several animals at several doses of a novel compound, and scoring animals according to their lever selection, the degree of substitution of the test stimulus for the training stimulus can be determined. Drugs which act through similar mechanisms will completely substitute for each other (at least 80% of the rats select the drug lever).

This procedure has been referred to by several names including tests for stimulus-substitution, stimulus-generalization, and stimulus-transfer. It is particularly useful in the development of SARs because the results allow the medicinal chemist to decide which analogues of a series are qualitatively similar, and then to estimate their potency ratios. It has been suggested that DD is the assay which most closely models the subjective experience of drug effects in humans (White, 1986; Rosecrans and Glennon, 1979). Various research groups have employed DD to develop SARs for derivatives of nicotine (Rosecrans et al, 1978), THC (Weissman, 1978), PCP (Browne, 1982), benzodiazepines (Shannon and Herling, 1983), and to assay drugs acting at excitatory amino acid receptors (Amrick and Bennett, 1987).

Glennon and coworkers have done extensive work on the SARs of hallucinogens (for reviews see Glennon et al, 1982a; Glennon et al, 1986) and have reported a high degree of correlation between human hallucinogenic potency and the DD-derived potency values for a series of 33 phenylalkylamine and indolealkylamine derivatives (Glennon et al., 1982a). However, one should keep in mind that since the accuracy of clinical potency estimates may be only ± 25%, correlation coefficients above 0.9 certainly lose their relevance (Dipaolo et al., 1978).

Finally, another important advantage in the DD paradigm is that various procedures can be employed to study the neuronal mechanisms mediating discriminative stimulus properties. Such studies utilize pharmacological manipulations, such as pretreatment with drugs which alter neurotransmitter levels or with specific antagonists. In this laboratory, DD has now been used to test compounds for LSD-like stimulus properties (Oberlender et al., 1984; Nichols et al., 1984a; Nichols et al., 1984b; Nichols et al., 1986a), and to compare the stimulus effects of hallucinogens, entactogens, and central stimulants (Nichols et al., 1986b; Oberlender and Nichols, 1988). Although labor and time investments are substantial, the useful advantages outlined above, especially when compared to alternative models, have justifiably earned the drug discrimination paradigm the reputation of "one of the most innovative approaches to studying the actions of drugs in animals" (Jacobs, 1987).

Brain Serotonin

Years of research into the mechanism of action of hallucinogens have resulted in the accumulation of extensive experimental data that implicate the involvement of serotonergic systems in the brain. However, there is much that remains to be learned about hallucinogenic drug action, although several hypothetical mechanisms have been proposed, tested and revised (see discussions by Heym and Jacobs, 1988; Davis, 1987; McCall, 1986). Freedman and Halaris (1978) described the source of difficulty encountered in studying the actions of hallucinogens this way: "The general problem is to deduce central activity, but the grammar and logic to do so have only begun to emerge with definition of the CNS physiology of 5-HT."

Thus, as more is learned about serotonergic systems, the mechanism of action of hallucinogens may become more clear. Conversely, the elucidation of the medicinal chemistry of hallucinogens may lead to valuable insight into the biochemistry associated with this important neurotransmitter. Some of the currently relevant concepts regarding serotonergic neuronal interactions with hallucinogens and entactogens are discussed in the following section.

Serotonin was first isolated about 40 years ago (Rapport et al., 1948). Its presence in the brain, and the ability of several analogues to influence mental activity, led to the suggestion that serotonin may function as a neurotransmitter (see references in Phillis, 1970). Woolley (1962) has reviewed the early work with serotonin, especially with regard to its involvement in mental disease and in the actions of hallucinogens. Chemically, serotonin is 5-hydroxytryptamine (5-HT, Figure 5).

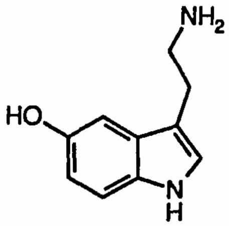


Figure 5. Molecular structure of serotonin (5-HT).

Serotonergic neurotransmission has been implicated in the control of numerous behavioral functions including pain, hunger, sex, emotion, sleep, memory and their associated pathological states (Tricklebank, 1985). The extremely complex oganization of the brain makes it difficult to learn exactly how such control is exerted. For example, serotonergic neurons are known to interact with a large number of other neurons, as well as with endocrine systems, such as the hypothalamic-pituitary-adrenal hormonal axis (Mellow et al., 1988; Koenig et al., 1988). The neuroanatomy and neurophysiology of serotonergic systems have been studied extensively and provide important clues to the functions of these neurons. The most striking physiological property of 5-HT neurons is their characteristic tonic firing pattern which may have a pacemaker or homeostatic function (Aghajanian and Wang, 1978).

The location of the cell bodies of neurons which use 5-HT as a neurotransmitter, along with their associated nerve terminals and the pathways in between, were originally mapped by histochemical methods and immunocytochemistry. These neurons originate from cell bodies in the raphe nuclei of the brainstem, and project fibers that innervate other parts of the brain stem, the spinal cord, and the forebrain (Aghajanian, 1984). Almost all central nervous system (CNS) centers receive serotonergic efferents but the innervation density varies considerably (Baumgarten and Lachenmayer, 1985).

The morphological features of these neurons allow the system to exert very generalized effects on the activity level of the innervated neurons. Serotonergic neurons can thus control CNS functions in a state-oriented rather than reflexive manner, with effects that are very subtle in nature (Baumgarten and Lachmayer, 1985). The firing rate and transmission characteristics of serotonin nerve cells are accurately controlled and adjusted depending on the state of arousal (Baumgarten and Lachenmayer, 1985).

Serotonin Receptors and the Mechanism of Action of Hallucinogens

As pharmacologists studied the effects of drugs on physiological systems, it was presumed that interactions between drug molecules and specific recognition sites or receptors in the target tissues were mediating these effects. There has been much interest in studying and classifying receptors through experiments involving the binding of compounds (ligands) that interact specifically with one type of receptor. In these binding studies, ligands containing a radioactive isotope are used since their amounts can be measured by counting radioactivity. The affinities of various compounds for that receptor are then measured by determining how well they displace the binding of the radioactive ligand.

Nutt and Linnoila (1988) have noted that the concept of a receptor involves a series of constructs that range from drug-receptor binding through effector (second messenger) systems to the molecular biology of the protein subcomponents. Substantial effort has been expended recently in order to identify specific types and subtypes of receptors. The identification of distinct receptors has been described (Peroutka, 1988) as a three part process. Initially, a "possible receptor" is identified as a unique radioligand binding site. The discovery of functional correlates of binding to this site by agonist molecules, whether endogenous or exogenous, allows the site to be referred to as a "probable receptor." Finally, a "definite receptor" is one which has been structurally identified. This consisits of the determination of its amino acid sequence which then allows it to be cloned, the product of which should be able to mimic both the radioligand and functional effects of the natural receptor (Peroutka, 1988).

On the basis of receptor binding experiments, Peroutka and Snyder (1979) concluded that at least two distinct 5-HT recognition sites exist in the CNS. 5-HT_1 receptors could be labeled with ^{3}H-5-HT, 5-HT_2 receptors could be labeled with ^{3}H-spiperone, and ^{3}H-LSD would label both. Numerous studies have now confirmed and extended these findings and attempts have been made to integrate these newer concepts with the classic work by Gaddum and Picarelli which established the "D" and "M" types of 5-HT receptors (see references in Bradley et al., 1986). In addition, the 5-HT_1 receptor has been divided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}, and a 5-HT_3 receptor has also been proposed. The characteristics of the various types of serotonin receptors are described in Table 1, which was reproduced from Peroutka, 1988.

The 5-HT_3 subtype has been well-studied in the peripheral nervous system but, until very recently, not in the CNS (Bradley et al., 1986; Peroutka, 1988). The 5-HT_{1A} site was demonstrated by Pedigo et al. (1981) to have high affinity for spiperone while a low affinity for this antagonist was observed for 5-HT_{1B} sites. The 5-HT_{1C} site was identified in the choroid plexus of various animal species by its high affinity for mesulergine but not ketanserin (see references in Hoyer, 1988) and has recently been cloned (see Peroutka, 1988). The 5-HT_{1D} site shares only a high affinity for serotonin with the other 5-HT_1 sites and has been shown to exist in all regions of bovine brain (see references in Peroutka, 1988). Although still the limiting factor of

Table 1. **Characteristics of serotonin receptor subtypes.**

	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$	5-HT$_{1D}$	5-HT$_{2}$	5-HT$_{3}$
Radiolabelled by	[^{3}H]5-HT [^{3}H]8-OH-DPAT [^{3}H]Ipsapirone [^{3}H]WB 4101 [^{3}H]Buspirone [^{3}H]PAPP [^{3}H]Spiroxatrine [^{3}H]5-MeO-DPAC [^{125}I]BH-8-MeO-*N*-PAT	[^{3}H]5-HT [^{125}I]CYP (rat and mouse only)	[^{3}H]5-HT [^{3}H]Mesulergine [^{125}I]LSD	[^{3}H]5-HT	[^{3}H]Spiperone [^{3}H]Mesulergine [^{125}I]LSD [^{3}H]Ketanserin [^{3}H]Mianserin [^{125}I]Methyl-LSD ?[^{3}H]DOB ?^{77}Br-R(−)DOB	[^{3}H]GR 65360 [^{3}H]ICS 205–930 [^{3}H]Quipazine [^{3}H]Zacopride
High-density regions	Raphé nuclei Hippocampus	Substantia nigra Globus pallidus	Choroid plexus	Basal ganglia	Layer IV cortex	Peripheral neurons
Potent pharmacological agents (i.e. <10 nM)	5-CT 8-OH-DPAT 5-HT RU 24969 (+)-LSD (−)-Pindolol	RU 24969 5-CT 5-HT (−)-Pindolol	Mesulergine Metergoline Methysergide	5-CT 5-HT Metergoline	Spiperone Mesulergine Methysergide Metergoline Mianserin Ketanserin	GR 65360 ICS 205–930 BRL 43694 Quipazine Zacopride MDL 72222
Second messenger	Modulation of adenylate cyclase	Inhibition of adenylate cyclase	PI turnover	Inhibition of adenylate cyclase	PI turnover	?
Membrane effects	Hyperpolarization via opening of K^{+} channels	?	Depolarization via opening of Cl^{-} channels	?	Depolarization	Depolarization
Other functional correlates	Basilar artery Thermoregulation Hypotension Sexual behavior 5-HT syndrome	'Autoreceptor'	?	?	Vascular contractions Platelet shape changes Platelet aggregation Paw edema Tryptamine seizures Head twitches	Transmitter release Bezold–Jarisch reflex

progress in this area, (Bradley et al., 1986) several 5-HT subtype-selective agonists and antagonists have been developed. For example, the 5-HT_{1A} site has been selectively labelled with [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) and the 5-HT_{1B} site with [^{125}I]-cyanopindolol (Frazer et al., 1988).

The 5-HT_{1C} site seems to share several structural and mechanistic characteristics with the 5-HT_2 receptor (Sanders-Bush and Breeding, 1988). Both have been demonstrated to be linked to the same transmembrane signaling pathway, the stimulation of phosphoinositide hydrolysis (Sanders-Bush and Breeding, 1988). The binding profiles of these two sites are also similar, except for serotonin's high affinity for the 5-HT_{1C} site (Conn and Sanders-Bush, 1987; Titeler et al., 1988). It is these two subtypes of serotonin receptors that appear to be most relevant to the pharmacological effects of hallucinogens, although the role of 5-HT_{1C} receptors is still unclear (Titeler et al., 1988).

Over the past five years, much evidence has accumulated supporting the idea that the behavioral effects of hallucinogens in rats may be mediated through actions at postsynaptic serotonin receptors (see references in White 1986). Most recently, since various 5-HT_2 agonists but not 5-HT_1 agonists substitute completely for LSD, the receptors mediating the cue properties of this and other hallucinogens have been proposed to be related to agonist activity at the 5-HT_2 subtype of serotonin receptor (Cunningham and Appel, 1987; Appel and Cunningham, 1986 and references cited therein).

Numerous subsequent studies have supported this conclusion. For example, LSD, DOM, and mescaline produce symmetrical substitution, and they all can be blocked by 5-HT_2 antagonists (Appel and Callahan, 1989, and references therein). Besides discriminative stimulus properties, a variety of other behavioral effects of LSD and mescaline are most effectively antagonized by compounds such as ketanserin and pirenperone which have a high affinity for 5-HT_2 sites (see references in Cunningham and Appel, 1987; Davis 1987). It is also noteworthy that LSD displays high affinity for all serotonin receptor subtypes, but only 5-HT_2 receptors display high affinity for LSD and other hallucinogens (Titeler et al., 1988; references in Heym and Jacobs, 1988).

Questions still remain concerning the exclusive role of 5-HT_2 agonist activity in the mechanism of action of hallucinogens. For example, Neilsen (1985) reported that 5-HT_2 antagonists do not block the LSD-cue in monkeys. Indeed, interspecies variation has very recently been demonstrated concerning the mediation of the disciminative stimulus properties of amphetamine which may involve different subtypes of dopamine receptors in the rat *versus* the rhesus monkey (Kamien and Woolverton, 1989). Unfortunately, no clinical studies regarding this issue have been published. In addition, there is evidence that 5-HT_2 agonist activity does not necessarily lead to hallucinogenic actions for all drugs. In fact, several non-hallucinogenic 5-HT_2 agonists, most notably quipazine, share discriminable properties with hallucinogens (White and Appel, 1982). The use of the DD assay as an animal model for hallucinogenic activity in humans may, therefore, have its caveats in the form of occasional false positives for other serotonin agonists. With that in mind, DD provides a good "first guess" in the prediction of clinical activity.

Colpaert et al. (1982) have emphasized the complexity of the interactions between serotonin antagonists and LSD, the latter displaying many characteristics of a mixed agonist/antagonist rather than a pure agonist at 5-HT receptors. Sanders-Bush et al., (1988) discussed the apparent contradiction in the results from experiments in which LSD has been observed to behave as a 5-HT_2 agonist and those where it acts as a 5-HT_2 antagonist. These workers found that both LSD and DOM are partial agonists at 5-HT_2 receptors and that the functional consequences of their actions depend on the concentration of endogenous serotonin. In the presence of low levels of serotonin, LSD will behave as an agonist; at higher serotonin concentrations, LSD would function as an antagonist (Sanders-Bush, et al., 1988).

Thus, the above discussion illustrates the complexity of the mechanism of action of hallucinogens. Although the 5-HT_2 receptor, at this time, seems to be most directly involved, in the absence of selective 5-HT_1 antagonists, and in view of the effectiveness of nonselective 5-HT antagonists in blocking the cues produced by hallucinogens, the involvement of 5-HT_1 receptors cannot be dismissed (Cunningham and Appel, 1988). A better understanding of the neurochemical correlates of the "psychedelic experience" may be facilitated by the recent use of additional hallucinogenic phenethylamine serotonin agonists, such as several 4-bromo- and 4-iodo-substituted analogues, in both *in vitro* and *in vivo* experiments (Titeler et al., 1987; Titeler et al., 1988; Mathis et al., 1988; Peroutka et al., 1988; Glennon et al., 1988; McKenna et al., 1989). In conclusion, the following comments of Heym and Jacobs (1988) seem appropriate:

> It would be premature to conclude that hallucinogens are "mind altering" drugs simply because they are potent 5-HT_2 agonists. Over thirty years of scientific inquiry has demonstrated that hallucinogens are very complex drugs which produce a multitude of effects in the central nervous system. Any of these actions may contribute to the cascade of neural events that results in a hallucinatory experience and it would be inappropriate to dismiss any one of these alterations as meaningless. Nevertheless, while the essential question regarding the sufficiency of 5-HT_2 agonism for hallucinogenesis presently remains unanswered, it appears that agonist activity at 5-HT_2 receptors is the prerequisite trigger for the psychedelic effect of classical hallucinogens.

Presynaptic Serotonergic Mechanisms and the Actions of Entactogens

When serotonin is released from its nerve terminals, its actions are terminated, to a large extent, by its reuptake into that terminal. This aspect of serotonin neurochemistry may relate to the action of entactogens which can effectively release serotonin and inhibit its reuptake into nerve terminals (see Chapter 4). Since a detailed discussion of the mechanism of action of entactogens is included in Chapter 4, it will only be noted at this point that while hallucinogens seem to exert their effects at postsynaptic serotonin receptors, entactogens probably act at presynaptic serotonergic sites.

CHAPTER 2 - AMIDES OF LYSERGIC ACID

Introduction

Much has been learned about the molecular structures of ergoline derivatives since the Lloyd brothers (1905) wrote that "chemistry applied to Ergot is productive of structural butchery." The alkaloids produced as metabolic products of the fungus *Claviceps* have been the focus of numerous chemical investigations leading to the identification of many of their structures and biosynthetic pathways (Floss, 1976). These compounds have also been studied intensively by pharmacologists interested in their broad spectrum of biological activity. From studies relating structure and activity, it was noted 30 years ago that small changes in the structure of ergot alkaloids produce surprisingly great changes in pharmacological effect (Votava et al., 1958).

Included among these compounds are various amides of lysergic acid. After Albert Hofmann synthesized the diethylamide of d-lysergic acid (LSD, 1, Figure 6) and discovered its hallucinogenic properties, a large number of structural modifications were investigated in SAR studies. The effect on activity of stereochemical variations at C-5 and C-8, saturation of the double bonds at C-9/C-10 and C-2/C-3, substitution at *N*-1, C-2 and *N*-6, and variations in the amide residue have been investigated (Hofmann, 1978). With some exceptions (e.g. Hashimoto et al., 1977; Hoffman and Nichols, 1985) very few new compounds have been prepared in SAR studies of hallucinogenic lysergamides in recent years.

Previous work regarding stereochemical aspects has demonstrated that changes in the (5*R*,8*R)* configurations of the two chiral centers of LSD (Leeman and Fabbri, 1959) results in loss of activity. The (5*S*,8*S*), (5*S*,8*R*), and (5*R*,8*S*) isomers were inactive in man as hallucinogens (Rothlin, 1957; Isbell et al., 1959). This demonstrates the high degree of stereospecificity in the ergoline ring system of the LSD molecule.

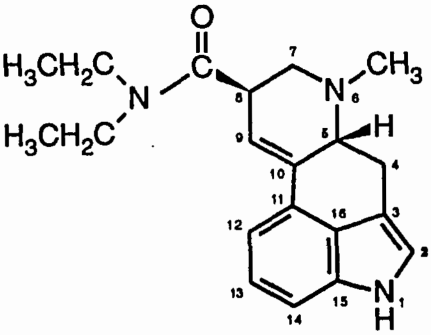


Figure 6. Ring numbering system for LSD, 1.

Of the many structural modifications to LSD that have been evaluated for their effect on activity over the last forty five years, very few have led to compounds with comparable potency. Only derivatives in which various alkyl groups replaced the methyl at the *N*-6 position of LSD seem to retain or even surpass the potency of the parent drug. Of these compounds, the n-propyl derivative was found to be equipotent to LSD, while the ethyl and allyl derivatives were more potent in the rat than the parent drug by factors of 1.6 and 2, respectively (Hoffman and Nichols, 1985). These DD-based estimates of potency were subsequently confirmed in preliminary studies with humans (A.T. Shulgin, cited in Nichols, 1986b). Thus, LSD can no longer be considered the most potent hallucinogen.

Numerous lysergic acid amides have been either isolated from natural sources or synthetically prepared and subsequently evaluated for hallucinogenic activity. None were found to have potency equal to or greater than that of LSD. It is the amide portion of lysergamides that is the focus of this chapter. In particular, efforts were directed toward elucidating the potential stereospecificity of the interaction between the biological receptor(s) and the achiral diethylamide moiety of LSD in its active binding conformation.

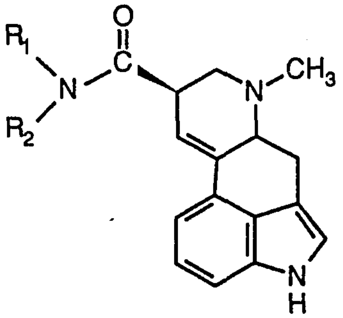
Amide Structure-Activity Relationship Studies of LSD

The most reliable source of information concerning the qualitative and quantitative effects of hallucinogenic drugs comes from experimentation with normal human subjects (Shulgin, 1978). There were several clinical studies of lysergamides in the late 1950s and early 1960s. From those early studies, involving a variety of amide derivatives, it was found that hallucinogenic activity is quite sensitive to molecular modifications of the diethyl substituents on the amide nitrogen of LSD (e.g. Abramson, 1959). The resulting decrease in potency was clearly demonstrated but the reason for this remains unclear. Shulgin (1981) has expressed the view that "the explicit requirement of the intact *N*,*N*-diethyl structure of the amide portion of LSD remains an unsolved enigma in this family of compounds."

In vitro SAR studies of lysergamides were conducted prior to the development of receptor binding techniques, and employed assays of peripheral serotonin antagonist (anti-serotonin) activity. Activity was determined by measurement of a compound's ability to reverse serotonin-induced contractions in isolated smooth muscle (i.e. rat uterus) preparations (Cerletti and Doepfner, 1958). Table 2 presents a summary of the results from these studies, along with the results of clinical evaluations of various lysergamides.

Peripheral anti-serotonin action on smooth muscle was, however, known at that time to have little correlation to effects in the CNS (Cerletti and

Table 2. Relative potency values for lysergic acid amides.



			Potency relative to LSD[a] From studies employing:	
R_2	R_3		Human[b]	*In Vitro*[c]
H	H	(Lysergamide)	3	4.3
Monoalkylamides:				
H	CH_3			6.3
H	CH_2CH_3		10	11.9
H	$CH_2CH_2CH_3$			40.0
H	$CH(CH_3)_2$			22.2
H	$CH(CH_2OH)CH_3$	(Ergonovine)	3	
H	$CH_2CH_2CH_2CH_3$			64.9
H	$CH_2CH_2CH_2CH_2CH_3$			75.1
Dialkylamides:				
CH_3	CH_3		10	23.2
CH_3	CH_2CH_3		3	
CH_3	$CH_2CH_2CH_3$		3	
CH_2CH_3	CH_2CH_3	(LSD)	100	100
CH_2CH_3	$CH_2CH_2CH_3$		32	
$CH_2CH_2CH_3$	$CH_2CH_2CH_3$		10	42.3
$CH(CH_3)_2$	$CH(CH_3)_2$			23.2
$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$			
Cyclic Amides:				
$-CH_2CH_2CH_2CH_2-$		(Pyrrolidide)	32	4.7
$-CH_2CH{=}CHCH_2-$		(Pyrrolinide)		4.1
$-CH_2CH_2CH_2CH_2CH_2CH_2-$		(Piperidide)		8.5
$-CH_2CH_2-O-CH_2CH_2-$		(Morpholide)	32	2.0

[a] Relative potency for both assays are expressed in terms of percent of LSD's potency. A value of 50 indicates one half as potent as LSD.

[b] Human potency values compiled by Shulgin (1981; 1982). References for the individual studies can be found in these reports.

[c] *In vitro* values represent serotonin antagonist potency as evaluated by Cerletti and Doepfner (1958) in the isolated rat uterus.

Doepfner, 1958). It was later specifically determined to be poorly correlated with LSD-like activity in humans (Isbell et al., 1959; Votava et al., 1958). In fact, compounds such as the N(6)-ethyl and allyl derivatives of norLSD, previously discussed as being more potent than LSD, were found to induce serotonin-like contractions in the isolated rat uterus, thereby complicating the determination of anti-serotonin potency (Hashimoto et al., 1977). Serotonin-like effects were also seen for several amide derivatives of LSD. The monoethyl amide and ß-hydroxy-diethylamide were reported to produce a maximum serotonin-like action similar to that of LSD but with decreased potency. It was concluded that the amide substituent contributes to the high affinity of LSD for serotonin receptors but may have little effect on intrinsic activity (Hashimoto et al., 1977).

Kumbar (1978) used molecular mechanics methodology in the study of the conformation of the amide substituent. Based on those data it was concluded that different modes of action were involved in the anti-serotonin and hallucinogenic effects of lysergamides. This is supported by the data for the cyclic amides listed at the bottom of Table 1. These compounds tend to elicit more potent hallucinogenic activity in humans than would be expected from their relatively low *in vitro* antiserotonin activity.

In addition, it was found that several monosubstituted cycloalkyl-lysergamides, in a series from *N*-cyclopropyl to *N*-cycloheptyl, elicited potent LSD-like antiserotonin effects *in vitro*, but lacked human hallucinogenic activity (Votava et al., 1958). Similar results were observed for 2-bromo-LSD (Isbell et al., 1959). Therefore, although anti-serotonin potency values form a rich data base for lysergamides, they are of questionable value in the study of hallucinogen SARs.

The exact nature of the pharmacological actions of hallucinogens at serotonin receptors has remained an area of intense interest over the years. Even today, the question of whether hallucinogens produce their characteristic effects through agonist and/or antagonist actions remains unresolved and suitable *in vitro* assays are still being developed (Pierce and Peroutka, 1989). Therefore, the focus of the data in Table 1 should be on the clinical values.

This presents a problem in that relatively few derivatives were clinically evaluated and, due to the nature of hallucinogenic activity, the data tend to be rather subjective. These values, therefore, lack a high degree of accuracy and should be viewed as rough estimates: 32 = 1/3 the potency of LSD; 10 = 1/10, 3 = less than 1/10 (Shulgin, 1981 and 1982). Despite these difficulties, clinical experiments with LSD derivatives have provided a good deal of useful information. Most striking is the observation that none of the lysergamides approach the potency of the parent compound; the diethyl amide seems to represent the optimum structure.

The ethyl amide (10) was found to be more potent than lysergamide itself (3), but ten times less potent than LSD (100). Longer monoalkyl substituents were not investigated in clinical experiments except for the 2-hydroxy-1-methylethyl amide (ergonovine), which was about as potent as lysergamide. The *n*-pentyl amide displayed significant anti-serotonin activity but was not evaluated in humans.

For the dialkyl amides, potency drops by an order of magnitude as a result of changing LSD's two ethyl groups by one methylene each to form either the dimethyl or di-*n*-propyl amides. The addition of a methylene to one ethyl group, as in the ethyl, *n*-propyl amide (32), attenuates potency to a lesser extent. However, the removal of one methylene, resulting in the methyl, ethyl amide (3), attenuates potency more than the replacement of the ethyl with a proton. Thus, the disubstituted amides are not necessarily more potent than the monosubstituted amides.

Two cyclized amides have been evaluated in humans. The pyrrolidide represents an LSD derivative in which an additional carbon-carbon bond connects the ß-carbons of the two ethyl groups. The morpholide can be viewed as a derivative in which these same carbons are bridged by an oxygen. Both of these compounds were about one third as potent as LSD.

These data illustrate clearly that hallucinogenic activity is highly sensitive to variations of the amide nitrogen substituents of lysergamides. Any change in the two ethyl groups of LSD results in a large decrease in potency. On the basis of these data, it seems safe to conclude that the amide substituent plays an important role in the hallucinogenic actions of LSD. Presumably, this relates to effects of the amide group on the binding interaction of the lysergamide with brain serotonin receptors (see Chapter 1). In view of the importance of the role of these receptors in a variety of psychopharmacological processes, a better understanding of the reasons for this observed sensitivity would be valuable.

There have been numerous attempts to develop equations (see Gupta et al., 1983 for review) that would explain the variation in biological activity in terms of quantitative structure-activity relationships (QSAR). Such theoretical approaches have the potential value of generating linear regression analyses that can predict the potency of new analogues and provide insight into mechanisms of action. Attempts to relate hallucinogenic activity to electronic parameters such as the energy of the highest occupied molecular orbital (E_{HOMO}) have not been as successful for lysergamides as they have for phenethylamine hallucinogens. However, hydrophobicity and steric factors appear to be important in all types of hallucinogenic drugs (Gupta et al., 1983).

Dunn and Bederka (1974), for example, found that the hydrophobicity of the amide substituent was important in determining anti-serotonin activity. However, these workers were forced to include a dummy variable in their equations in order to account for the low *in vitro* potency of cyclic amides. Interestingly, no relationship between structural parameters and human hallucinogenic potency could be found. The latter was attributed more to the paucity of clinical data than to the lack of existence of such a relationship.

Other parameters that were developed include molecular connectivity (Glennon and Kier, 1978) and van der Waals volume (Gupta et al., 1981). While the former was analyzed for lysergamides only with respect to serotonin antagonist activity, the van der Waals volume (V_W) was found to be significantly related to hallucinogenic activity. However, only lysergamides with alkyl groups no larger than ethyl, in addition to three cyclic amides, were examined. The reported equations admittedly did not provide any deeper insight into hallucinogenic actions (Gupta et al., 1981). In fact, none of the QSAR studies revealed parameters which could generally provide good correlations with hallucinogenic activity or significant insight into the reason for the unique effect of LSD's diethyl group. Gupta et al. (1983) concluded that the shortcomings of QSAR studies with hallucinogens have limited their success and they caution against a heavy reliance on the results of this approach.

More qualitative SAR methods have also been applied to hallucinogen SAR. A search for possible structural analogies between various hallucinogens and serotonin, have been proposed (see references in Andrews and Lloyd, 1986) in attempts to relate molecular variables with activity, but conclusive arguments have yet to be made. It is difficult to imagine how the amide portion of LSD could correspond to any part of the serotonin molecule as it would occupy a receptor (Dunn and Bederka, 1974). Since the entire carbon skeleton of the serotonin molecule can be viewed as being contained within the ergoline nucleus of LSD, it is not readily apparent why or how the substituent on C-8 should have a significant effect on activity.

One explanation that has been discussed in the literature relates to the effect of the amide on the conformation of the ergoline D ring. For example, it has been noted that the replacement of the carboxamide substituent with methyl at position 8 leads to a loss of the "preferred" conformation and a concomitant loss of hallucinogenic activity (see Baker et al., 1973). If no substituent is present, i.e C-8 is a CH_2, the LSD-like activity probably is abolished, although one report suggests that this may not be the case. This analogue, descarboxylysergic acid, was synthesized by Bach et al. (1974) and seemed to retain LSD-like activity in a simple mouse assay, suggesting that the 8-substituent is not necessary. However, the lack of LSD-like DS properties of descarboxylysergic acid (Oberlender and Nichols, unpublished data) argues strongly against this view.

Hallucinogenic activity is restored, however, even when a primary amide substituent is present at C-8. Lysergamide, a naturally occurring component of morning glory seeds, elicits characteristic hallucinogenic effects in humans, although its potency is less than one tenth that of LSD (see Table 1). Therefore, the amide moiety seems to be essential for hallucinogenic activity and the diethyl group somehow exerts an optimal influence.

Rationale for the Selection of Target Compounds

What could possibly be so special about a simple diethyl amide group? Pharmacokinetic factors could account for some of the differences in potency. However, the physicochemical properties of the simple alkyl substituents, discussed in the previous section, can be expected to be quite similar to those of the diethyl substituent. This suggests that pharmacodynamic factors, such as the influence of the amide substituent on the drug-receptor interaction, must also be important. Obviously, the relative size of the alkyl substituents may be expected to influence this interaction. Perhaps less obvious is the potential for substituents of equal size to affect activity differently due to stereochemical factors.

Since the diethyl groups of LSD have rotational freedom, it seems reasonable to consider that adoption of a specific conformation, the biologically active one, may somehow facilitate drug-receptor interactions associated with the observed effects. Thus, the dramatic attenuation of activity observed for a variety of lysergamides relative to LSD could also be related to differences in the spatial arrangements adopted by the alkyl groups in the amide moiety.

If the free rotation of the diethyl groups could be restricted so that conformational properties become relatively fixed, it should be possible to demonstrate the influence of these stereochemical factors on activity. An effective method for studying "frozen" geometries in molecules involves the use of rigid analogues. Thus, the primary goal of the work described in this chapter was to synthesize and evaluate several rigid LSD analogues anticipating that data might be obtained that could help elucidate the influence of conformational variables in the diethyl amide substituent on hallucinogenic activity.

As discussed in Chapter 1, hallucinogenic activity in humans is usually reflected in LSD-like discriminative stimulus properties. Therefore, the drug discrimination paradigm was employed as a means of evaluating these rigid analogues in rats. The results of these studies were expected to demonstrate potency differences for the individual compounds in LSD-substitution tests. If conformational factors associated with the ethyl groups exert a critical influence on activity, the analogue which most closely resembles the stereochemistry of the biologically active LSD conformer should be identifiable as the most potent test drug.

The isomers of lysergic acid-(2,3-dimethylaziridinyl)amide, 2, 3, and 4 (Figure 7), were selected as useful target compounds. These derivatives have a molecular weight and size that is very similar to the diethyl amide. The 2,3-carbon-carbon bond of the aziridine ring represents the only molecular modification that has been introduced to the parent structure. As a result, these novel LSD analogues contain two additional chiral centers, resulting in three diastereomeric forms: (*R*,*R*)-*trans*, 2, (*S*,*S*)-*trans*, 3, and *cis*, 4.

In addition, 2 and 3, could be synthesized from *trans*-2,3-dimethylaziridine, and it was considered possible that they could then be resolved as diastereomers. The *cis*-meso-isomer, 4, derived from *cis*-2,3-dimethylaziridine, is shown in Figure 7 in one of its two conformations which are interconvertable through nitrogen inversion. The pyrrolidide (5, Figure 7) was also included.

The pyrrolidide is identical in molecular weight to the lysergyl-dimethylaziridines and represents an analogue of LSD in which the terminal methyl groups of the the diethyl substituent are connected by an additional bond. A similar bond connects the methylene carbons of LSD's ethyl groups in 2, 3, and 4. Thus, the comparison of the potency of 5 relative to the novel analogues was seen as valuable. In addition, since 5 has been studied previously for hallucinogenic activity (Murphree et al., 1958), it provides an additional means of comparing the results from the DD assay with those from human studies.

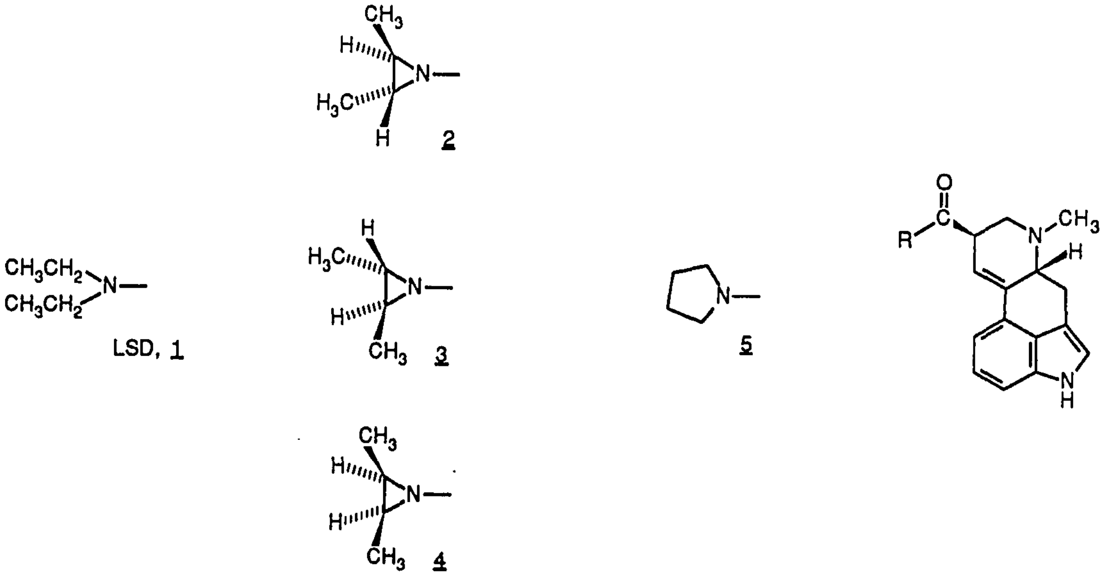


Figure 7. Molecular structures of target compounds.

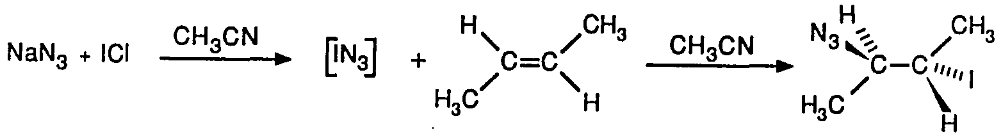
It was anticipated that the molecular distortion imposed by the three-membered ring could result in some degree of compromise in the use of a 2,3-dimethylaziridinyl amide to model a diethylamide. For example, the presence of ring strain in aziridines results in the formation of bent bonds which are high in *p*-character (Padwa and Woolhouse, 1984). The normal C-N-C bond angle in aliphatic amines of 111° is drastically reduced to 60° in aziridines and several methods have demonstrated considerable C-C bond shortening (1.48 Å vs. 1.54 Å) and C-N bond lengthening (1.49 Å vs 1.46 Å) for aziridines compared with non-cyclized dialkylamines (Padwa and Woolhouse, 1984).

In addition, the sp^2-hybridization of the atoms in the aziridine ring causes the 1-acylaziridine nitrogen to be more pyramidal, relative to simple amides (Anet and Osyany, 1967; Zacharias and Trefonis, 1968). The resulting increase in the resistance of *N*-acylaziridines to adopt a planar amide conformation prevents typical electron resonance and makes them resemble ketones more than amides (see references in Bucholz and Stamm, 1986). However, these electronic and steric factors are common to all three analogues, 2-4.

Therefore, since hallucinogenic activity is very sensitive to the amide substituent, conformational differences among these stereoisomers may be expected to contribute significantly to relative potency in eliciting LSD-like DS effects.

Synthesis and Identification of Products

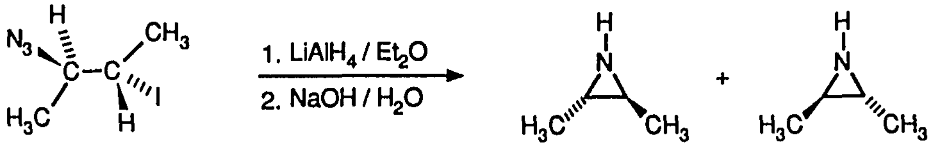
The synthesis of *trans*-2,3-dimethylaziridine, 11, was accomplished in two reactions. *Trans*-2-butene, 9, was reacted with iodine azide, 8, by the method of Fowler, et al. (1967). Iodine azide, highly explosive in its isolated state, is formed *in situ* by reacting sodium azide, 6, with iodine monochloride, 7, in acetonitile. Subsequent addition of 9 leads to the formation of *erythro*-2-azido-3-iodobutane, 10, in excellent yield. The workup removed all unreacted salts resulting in pure 10 without the need for additional purification.



6 7 8 9 10

Figure 8. Synthesis of *erythro*-2-azido-3-iodobutane.

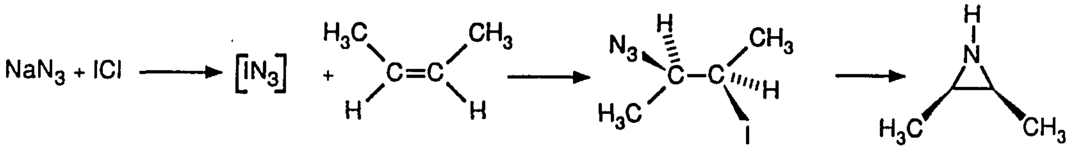
The stereospecific synthesis of *trans*-2,3-dimethyl aziridine, 11 (Figure 9), was then accomplished by lithium aluminum hydride reduction of 10, followed by base-catalyzed ring closure (Hassner et al., 1969).



10 (*S*,*S*)-11 (*R*,*R*)-11

Figure 9. Stereospecific synthesis of *trans*-2,3-dimethylaziridine.

The synthesis of *cis*-2,3-dimethylaziridine, 14, was accomplished by an identical procedure except for the use of *cis*-2-butene, 12. Reduction of *threo*-2-azido-3-iodobutane, 13, afforded the desired product (Figure 10).



6 7 8 12 13 14

Figure 10. Stereospecific synthesis of *cis*-2,3-dimethylaziridine.

The final synthetic step involved the amide-forming condensation of these amines with lysergic acid. Several potential complications of this reaction were considered. The classical methods for preparing amides by acylation of amines with esters or acid chlorides were known to fail when applied to lysergic acid (Garbrecht, 1959). In addition, the three-membered ring leads to several unusual characteristics for the reactivity of azirdine bases relative to that of open chain amines. Fortunately, the former retains the reactivity of the latter in the nucleophilic attack of carbonyls, an essential part of the amide forming reaction.

Fedor et al. (1966) noted that although the pK_a of aziridine (8.04) is lower than that of dimethylamine (10.64), it demonstrates a nucleophilicity in reacting with ester carbonyls anticipated for an amine with a pK_a of about 11. This hypernucleophilicity of aziridines has been suggested to result from the lessened crowding in the formation of the transition state due to the C-N-C bond angle constraint (Fedor et al., 1966). Thus, although electron delocalization in the three-membered ring may decrease basicity, thus lowering the pK_a, steric factors, of little importance to protonation, tend to compensate with respect to nucleophilicity.

Therefore, the formation of the aziridinyl lysergamides was anticipated to occur under conditions which have previously proven successful in the synthesis of LSD. Initially, the condensation was attempted using the practical and efficient method of Johnson et al. (1973), shown in Figure 11. Lysergic acid, 15, and a 9-fold excess of amine were stirred under nitrogen, in refluxing chloroform. The heat was removed and $POCl_3$, 16, was added dropwise over 5 minutes. While this procedure was employed without difficulty with pyrrolidine, in the synthesis of the pyrrolidide, 5, the desired amides were not isolated when the isomers of 2,3-dimethylaziridine were used (e.g. 14 in Figure 11). In the latter case, reactions other than the intended one seemed to have occurred. Extension of the reaction time, as well as the addition of an extra 5 equivalents of amine, had no observable effect on the outcome. 1-Phosphorylated aziridines are known to give respectable yields of ring opened products upon treatment with carboxylic acids (Dermer and Ham, 1969).

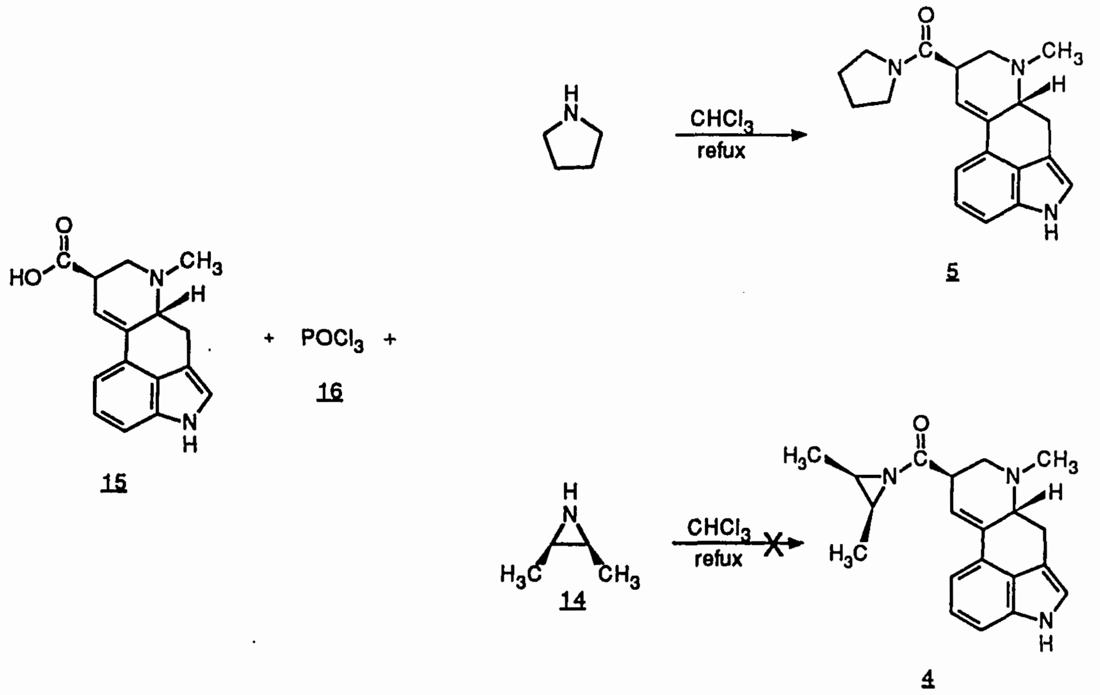
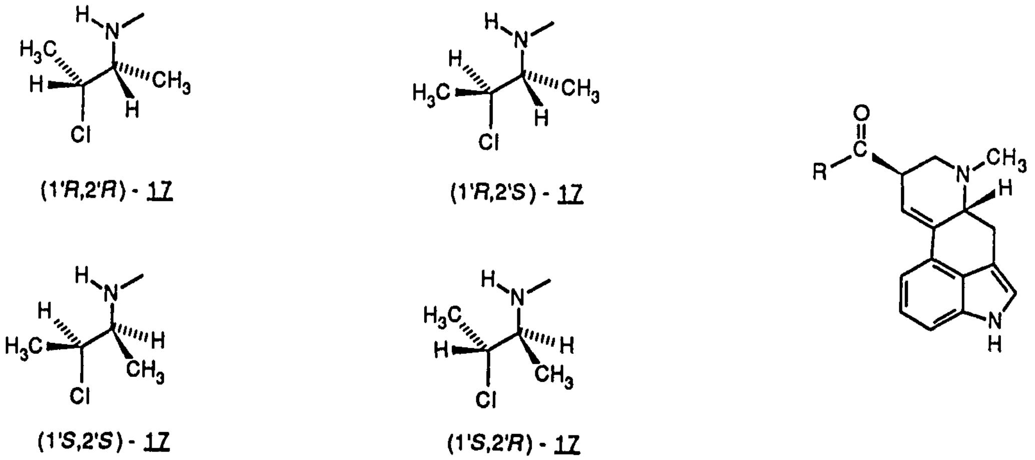


Figure 11. Lysergamide synthesis with phosphorous oxychloride.

Several other synthetic procedures that had been used successfully by others in the synthesis of LSD were attempted. Various coupling reagents such as *N*,*N*-carbonyl diimidazole (Cerny and Semonsky, 1962), trifluoroacetic anhydride (Pioch, 1956), and a triphenylphosphite-imidazole complex (Nakahara and Niwaguchi, 1974) were employed. The products isolated when all of these procedures were applied to the synthesis of the aziridinyl amides were complex mixtures, with no evidence of formation of the lysergylaziridines.

The alternative procedure of Johnson et al. (1973), in which phosphorus oxychloride and the amine were added simultaneously to a chloroform suspension of lysergic acid at reflux, was observed to produce a relatively clean product. However, it was determined that the purified isolated products were not the desired lysergylaziridines. The potential isomerization of the product to a lysergyl-(2-oxazoline) or, more likely in the case of the 2,3-dimethylaziridinyl amides, to the *N*-allyl amide (Bieron and Dinan, 1971) was considered. The analytical data were inconsistent with these potential products as well. After a simple Beilstein test demonstrated the presence of halogen, the products were ultimately identified as isomers of *N*-(2-chloro-1-methylpropyl)-lysergamide (17, Figure 12).



(1'*R*,2'*R*) - 17

(1'*R*,2'*S*) - 17

(1'*S*,2'*S*) - 17

(1'*S*,2'*R*) - 17

Figure 12. Molecular structures of the isomers of 17.

Data from analyses utilizing ^{1}H-NMR, ^{13}C-NMR, IR, and high resolution mass spectrometry were all consistent with this structural assignment. Figure 13 illustrates the 200 MHz ^{1}H-NMR spectra of LSD and the analogues synthesized. Chemical shift assignments and coupling constants can be found in Table 5 (page 70). The amide protons of the isomers of 17 absorb in the aromatic region, between the $CHCl_3$ peak at 7.25 ppm and the indole NH peak at about 8 ppm. The chemical shifts of the protons on the aromatic carbons (7-7.5 ppm), and those on C-2 (about 6.9 ppm) and C-9 (about 6.4 ppm), are similar to those observed for LSD, 5, and other lysergamides (Bailey and Gray, 1972). Two doublets, one for each of the two methyl groups in the *N*-alkyl substituents are seen in the upfield region (1-1.5 ppm). Figure 14 shows the expanded region of the proton NMR from 2.5 to 4.5 ppm, where the other ring protons and *N*-methyl protons (about 2.5 ppm) absorb. The chemical shifts of the *N*-alkyl methine protons of the isomers of 17 are between 4 and 4.5 ppm.

The proton-decoupled ^{13}C-NMR spectra of the lysergamides are shown in Figure 15 and the chemical shift assignments for these compounds, together with those for LSD and 5, are listed in Table 6 (page 71). The data for the isomers of 17 are consistent with the *N*-(2-chloro-1-methylpropyl)-amide structure. The *N*-alkyl carbon *alpha* to the nitrogen is observed at about 49 ppm, a downfield shift of 10 ppm relative to the α-carbons of LSD. This difference is consistent with the predicted shifts of *tertiary vs. secondary* carbons (Whitesell and Minton, 1987). The chemical shifts of the CH_3 carbon *gamma* to the nitrogen (about 22 ppm), and of the CH carbon bonded to the chlorine atom (about 60 ppm), are in agreement with the observed effects of chlorine reported in the literature (Nouguier et al., 1981). The CH_3 carbon ß to the amide nitrogen is observed at about 14 ppm for 17-1 and 17-2, and at about 19 ppm for 17-3 and 17-4.

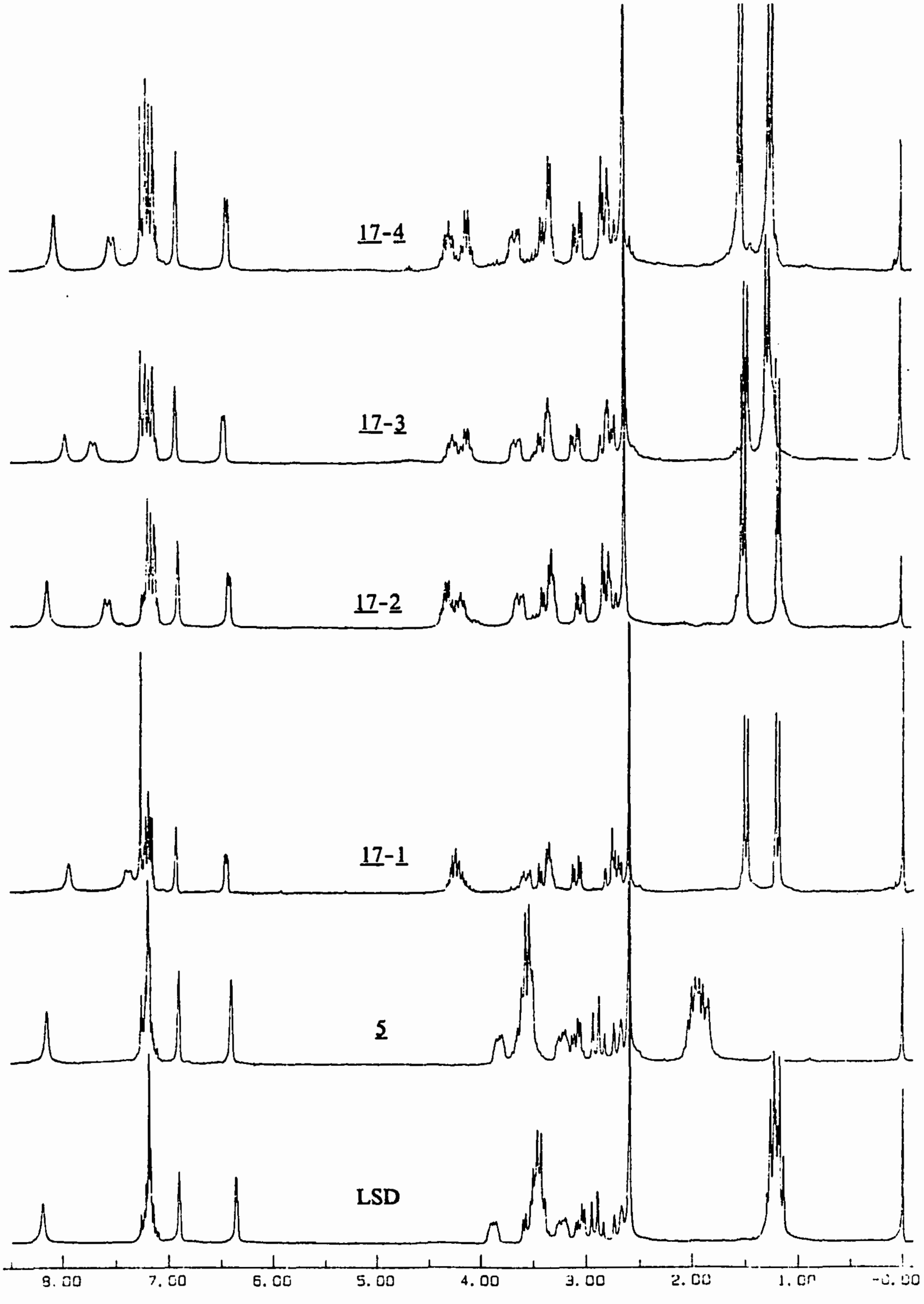


Figure 13. ^{1}H-NMR spectra of lysergamides.

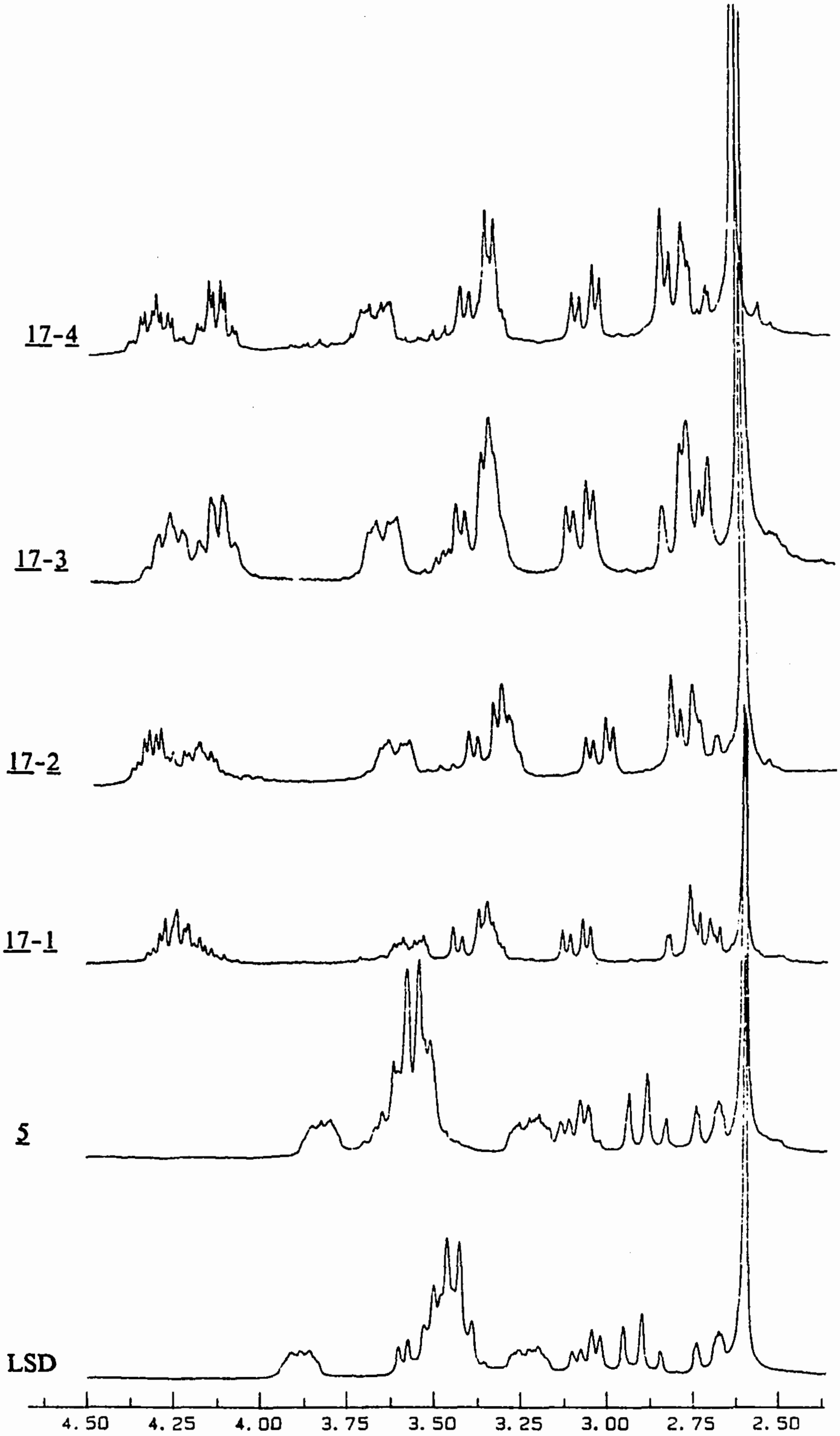


Figure 14. ^{1}H-NMR spectra of lysergamides expanded in the 2.5-4.5 ppm. region.

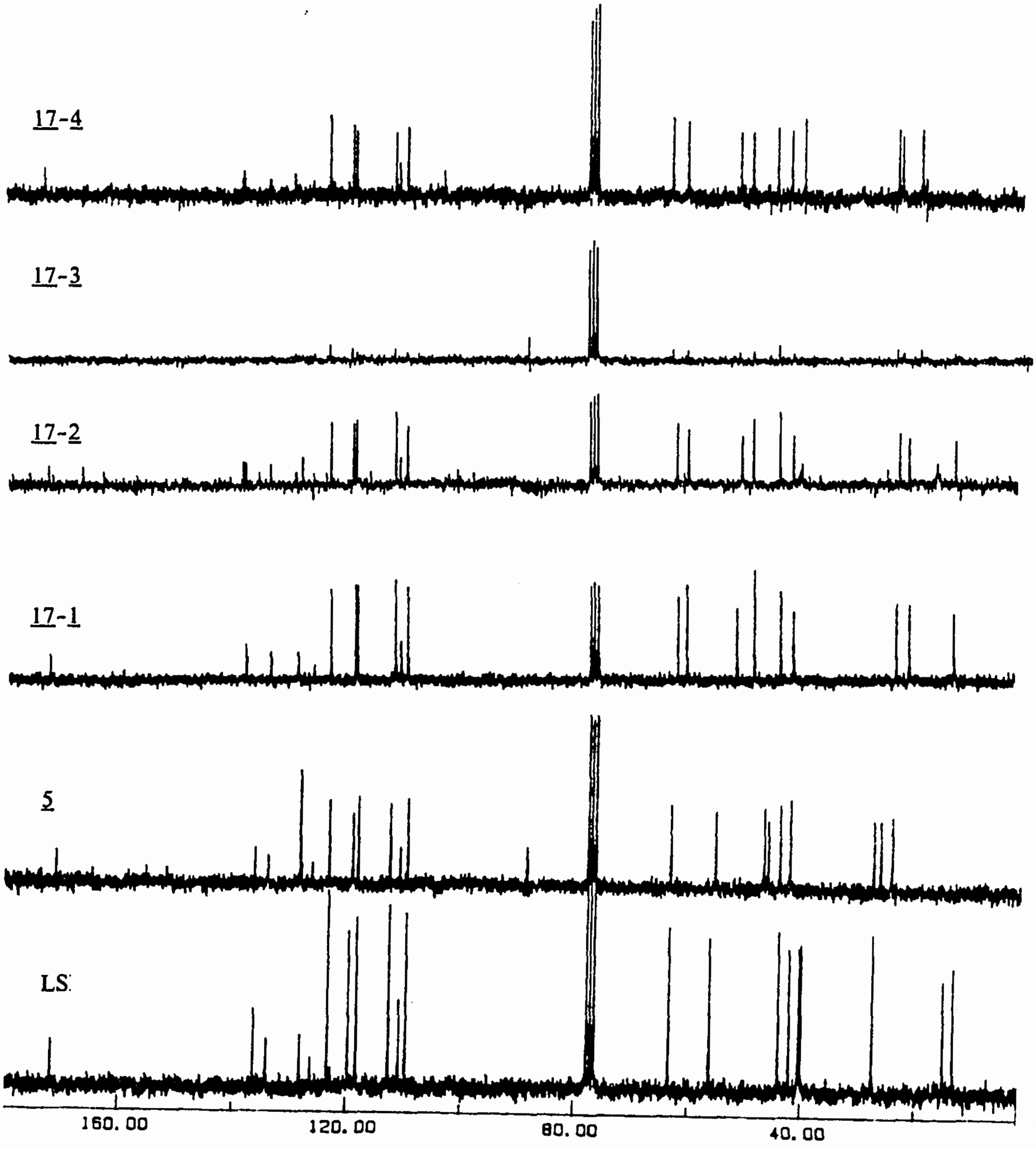


Figure 15. ^{13}C-NMR spectra of lysergamides.

A *gamma*-chlorine substituent in this type of alkyl group reportedly induces a conformation-dependent upfield shift in the ^{13}C-absorbance (Nouguier et al., 1981). The difference in the chemical shifts of this carbon for the isomers of 17 may, therefore, reflect conformational differences that exist in $CDCl_3$.

High resolution chemical ionization mass spectrometry analysis of the isomers of 17 determined the molecular weight to be 357.1638 which is within experimental error limits of the calculated value, 357.1686. The fragmentation of the molecular ion resulted in loss of HCl for all of the isomers. Finally, infrared absorbances, detailed in the experimental section, included an absorption band at 1650 cm^{-1}. This carbonyl stretching frequency is diagnostic of a secondary amide and provides additional evidence that these compounds are not acylaziridines. The latter compounds show a characteristic shift of the carbonyl absorbance to about 1720 cm^{-1}, due to the lack of amide resonance (Katritzky and Taylor, 1971).

The formation of the isomers of 17 may have resulted from the nucleophilic ring opening of the desired *N*-acylaziridine by attack of chloride ion, generated during the reaction (Dermer and Ham, 1969). Similar reactions have been reported previously. For example, when HCl was bubbled into a solution of 1-benzoylaziridine, chloride ion attack on the ring produced *N*-(2-chloroethyl)-benzamide (Fanta and Deutsch, 1958). In addition, Pettit et al. (1967) found that HCl readily opened the aziridine ring of *N*-(3,4-dichlorobenzoyl)-aziridine, in $CHCl_3$, affording a quantitative yield of *N*-(2-chloroethyl)-3,4-dichlorobenzamide in only 5 minutes at room temperature.

The synthesis of 1-acylaziridines is known to be complicated by the high reactivity of the product to nucleophilic ring opening. This is due to the ability of the carbonyl to delocalize the increasing electron density of the nitrogen atom as the ring opens, greatly reducing the activation energy needed for a nucleophilic reaction (Olah and Szilagyi, 1969; Ham, 1964). As a result, the S_N2-like ring-opening reactions of these "activated aziridines" (Ham, 1964) with nucleophiles occurs even in the absence of an acid catalyst (see references in Olah and Szilzgyi, 1969).

In the presence of excess aziridine, the protonated acylaziridine would exist only at very low concentrations. However, acid protonation of the *N*-acylaziridine leads to "double activation" and a drastically increased reactivity toward nucleophilic ring-opening (Bucholz and Stamm, 1986). Olah and Szilagyi (1969) found that *N*-acylaziridines could be protonated without ring opening only under special conditions.

Therefore, the formation of 17 can be readily rationalized under the conditions which existed in the condensation. Model reactions were performed in order to investigate further the findings described above.

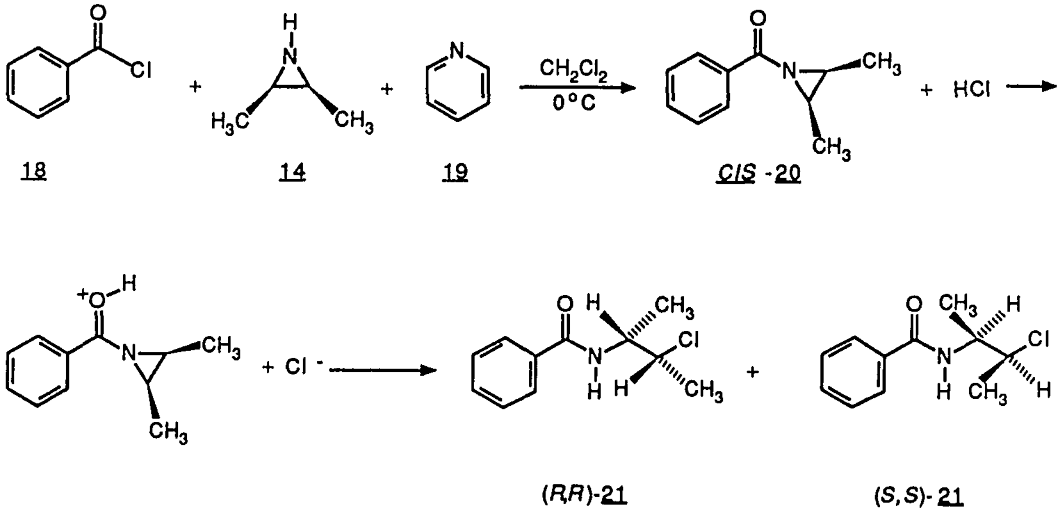


Figure 16. Attempted synthesis of *N*-benzoyl-2,3-dimethylaziridine.

Thus, 2,3-dimethylaziridine (e.g. the *cis*-isomer, 14, in Figure 16) was reacted with benzoyl chloride, 18, in the presence of pyridine (19) as an HCl scavenger. Similar to the lysergamide reaction, the products were identified as isomers of *N*-(2-chloro-1-methylpropyl)-benzamide, 21. *N*-Benzoylaziridine, 20, could not be isolated from the reactions starting with either *cis*- or *trans*-2,3-dimethylaziridine. Therefore, chloride attack of *N*-benzoylaziridine may have occurred in this model system also.

The identification of 21 was verified by comparing its analytical data with those of the same compound prepared by a different synthetic route (see Figure 17). *Cis*-2-epoxybutane, 22, was treated with benzyl diethylaluminum amide (Overman and Flippin, 1981) affording *threo*-3-(*N*-benzylamino)-2-butanol. Debenzylation by catalytic hydrogenolysis yielded *threo*-3-amino-2-butanol, 23, which was then condensed with benzoyl chloride, 18, in CH_2Cl_2 with triethylamine. The resulting alcohol was treated with triphenylphosphine and carbon tetrachloride, resulting in formation of the chloride, 21.

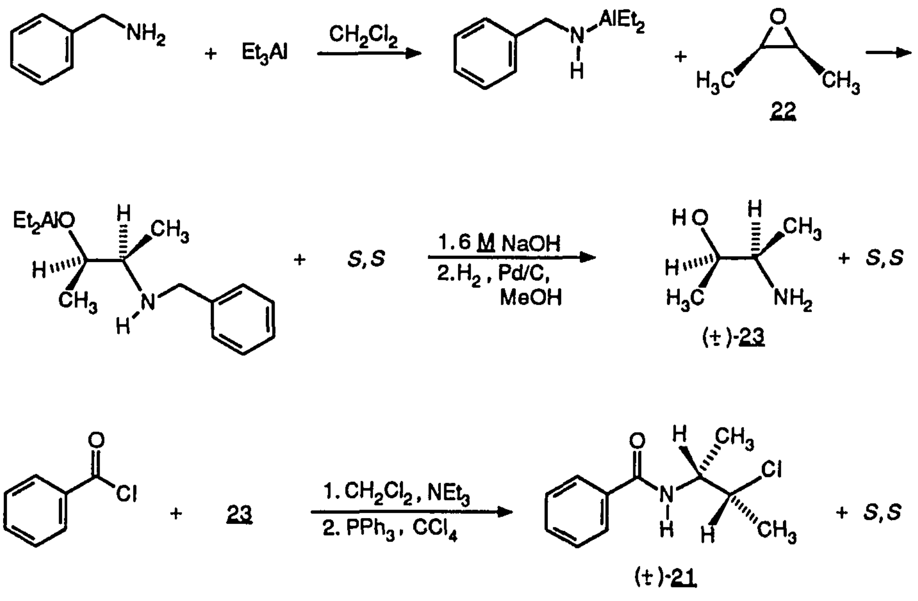


Figure 17. Synthesis of *N*-(2-chloro-1-methylpropyl)-benzamide, 21.

Comparison of the ^{1}H-NMR spectra suggests that the product obtained from this synthesis (Figure 18, A) is identical to the product obtained from the reaction (see Figure 16) of benzoyl chloride with the *cis*-isomer of 2,3-dimethylaziridine (Figure 18, B) and not the product of the same reaction using the *trans*-isomer (Figure 18, C).

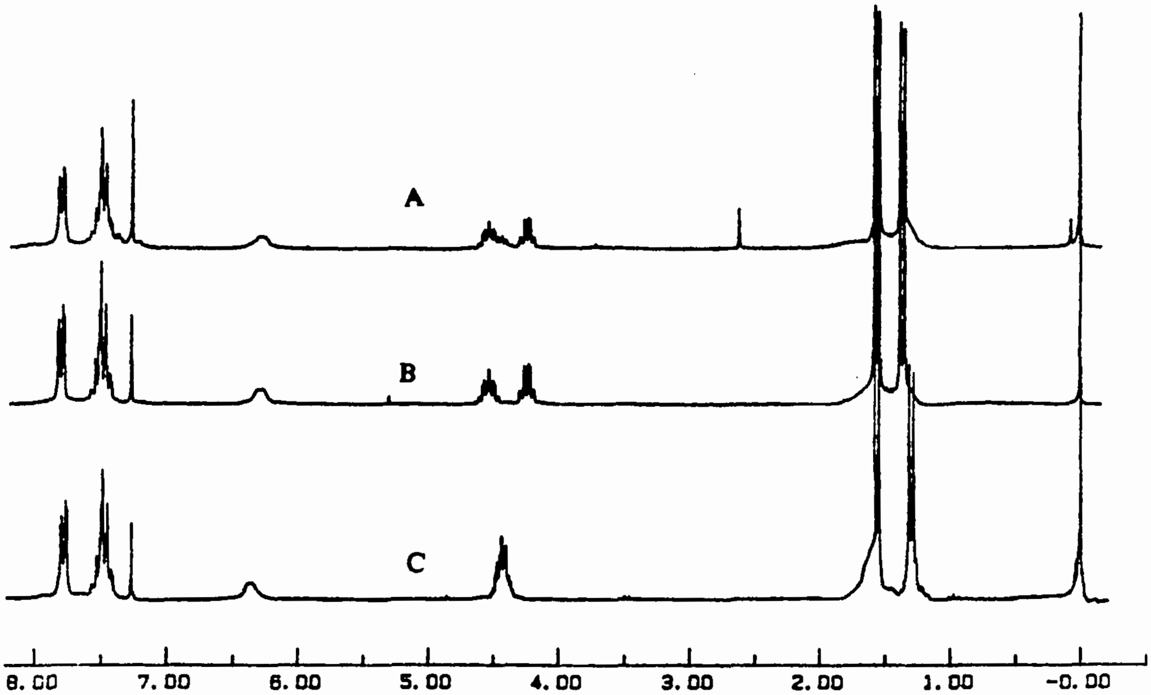


Figure 18. ^{1}H-NMR spectra of benzamide derivatives.

Parallel differences were observed in the NMR spectra of the lysergamides derived from *cis*- and *trans*-2,3-dimethylaziridine and the benzamides derived from the same bases. A comparison of the absorptions for the analogous protons of the *N*-alkyl substituents were identical except for the chemical shifts of the broad peaks assigned to the amide protons. The upfield position of these peaks (at 6.4 ppm) for the benzamides, relative to the lysergamides, may be due to differences in H-bonding. Therefore, the analytical data described above can be seen as strong support for the structural assignments of the stereoisomers of 17, obtained in the condensation of lysergic acid with *cis*-

and *trans*-2,3-dimethylaziridine. However, the assignment of stereochemistry to the products isolated cannot be made definitively.

A comparison of the chemical shifts of the protons on the methyl carbon that is ß to the amide nitrogen, and the line shapes of the methine proton peaks, with NMR data reported for a similar compound (Lessard et al., 1981), suggests that the *threo*-isomer results when the starting amine is *cis*-2,3-dimethylaziridine, and the *erythro*-isomer is obtained when the *trans*-amine is used. Although this assignment is not definitive, it is consistent with a nucleophilic ring opening of the acylaziridines by chloride ion. Based on the known reactivity of *N*-acylaziridines, a possible mechanism for the formation of *N*-(2-chloro-1-methylpropyl)-amides is described below in Figure 19.

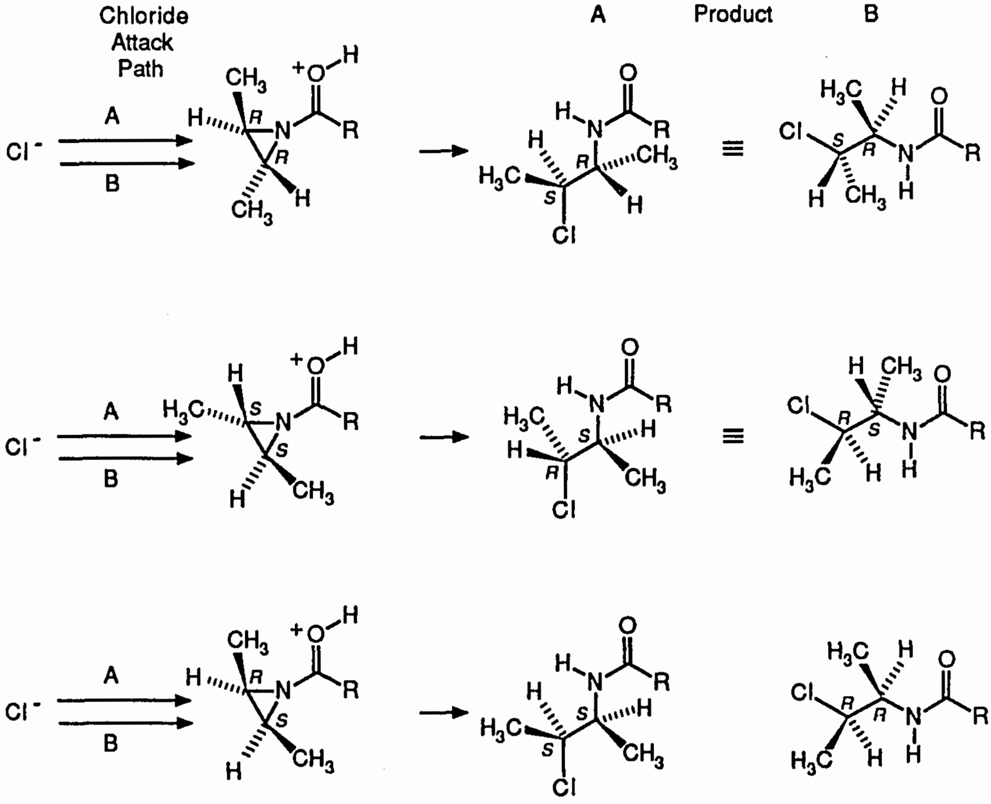


Figure 19. Acid catalyzed nucleophilic ring opening of acylaziridines by chloride ion.

According to this mechanism, since chloride attack can occur at either aziridine carbon (path A or B in Figure 19), one isomer of product is formed from each of the (*R*,*R*)- and (*S*,*S*)-*trans*-2,3-dimethylaziridinyl lysergamides, 2 and 3. Thus, this reaction may be predicted to form a mixture of *erythro* isomers, (1*R*,2*S*)-17, and (1*S*,2*R*)-17. Chloride attack of the *cis*-meso-amide, 4, (only one conformer shown in Figure 20) forms a mixture of *erythro*-isomers, (1*R*,2*R*)-17 and (1*S*,2*S*)-17. Two isomers were isolated in the reaction of lysergic acid with *trans*-2,3-dimethylaziridine, and two with the *cis* isomer. In both cases, the individual isomers were separated chromatographically. After workup, the normal isomers were separated from the isolysergic acid amides by centrifugal chromatography (chromatotron) on an alumina/ silica plate as described previously (Hoffman and Nichols, 1985). The two diastereomers that were formed in each of the reactions were separated using a silica chromatotron plate and eluting with ethyl acetate for the isomers obtained from 11, and elution with 5% Et_2O/CH_2Cl_2 for those from 14.

Unambiguous assignment of absolute configurations would be necessary in order to determine which of the four isolated products corresponded to which configuration. Unfortunately, attempts to grow crystals of various salts of these compounds, suitable for X-ray crystallography analysis, were not successful. For the purposes of the present study, the compounds were referred to on the basis of elution order in the second chromatotron step. Thus, the first isomer eluted from the reaction of *trans*-2,3-dimethylaziridine was temporarily labelled as 17-1, and the second, 17-2. These products are referred to in the experimental section as the *erythro*-isomers. Similarly, from the reaction of the *cis*-amine, the products were labelled 17-3 and 17-4, and tentatively referred to as the *threo*-isomers.

As the high reactivity of *N*-acylaziridines became increasingly apparent, concern increased regarding the successful completion of the project with the original target molecules. The necessity of acidifying the lysergamides, in order to prepare water soluble salts suitable for pharmacology studies, may present a significant hazard to the integrity of the aziridine ring. Even if the compounds remained intact long enough to be injected into rats, it seemed likely that a ring-opening attack by biological nucleophiles would occur at physiological pH before the compounds could accumulate in the brain. Therefore, the high reactivity of *N*-acyl aziridines that is useful synthetically (Pettit, et al., 1967), may create significant problems regarding their *in vivo* use.

It was decided instead to evaluate the chloro compounds, 17, for biological activity. The original goal of the project could still be achieved, since 4 stereoisomers had been prepared. Although these derivatives do not model the diethyl substituent, their evaluation could be expected to indicate whether the amide substituent bound to a stereochemically demanding site at the receptor. The final products, 5 and 17-1, -2, -3, and -4 were converted into their *l*-tartrate salts and dissolved in saline in appropriate concentrations. Substitution tests were then performed in LSD-trained rats as described in Chapter 1.

Evaluation of LSD-like Discriminative Stimulus Properties

The results of the drug discrimination testing are given in Table 3. The experimental data can be found in Table 15 in appendix B. The four isomers of 17, and the pyrrolidide 5, completely substituted for LSD. However, the potency of the test compounds varied over a 22-fold range. The least potent compound was 17-4 and 17-1 was found to be more potent than LSD itself. All 8 rats tested with saline selected the saline lever.

Table 3. Potency of lysergic acid amides in LSD-trained rats.

Compound	ED_{50} (μM/kg)	95% C.I.	Potency Relative to LSD
17-1	0.027	0.017-0.044	155
LSD	0.042	0.025-0.072	100
17-3	0.156	0.079-0.308	27
5	0.168	0.115-0.247	25
17-4	0.387	0.275-0.544	11
17-2	0.605	0.324-1.130	7

Based on the 95% confidence intervals, the six ergolines in Table 3 can be separated into three groups of two compounds. The high potency group includes LSD and 17-1. A relatively moderate potency was observed for 17-3 and 5, while 17-4 and 17-2 were considerably less potent in producing LSD-like discriminative stimulus properties. In terms of disruptive effects (see Chapter 1), none of the test compounds produced large numbers of disruptions at stimulus-generalization doses. The compound which produced the highest degree of disruptive effects was 5 (31% of the rats tested with the 0.3 μM/kg dose did not finish 50 presses in 5 minutes).

Conclusions

The results suggest that stereochemical differences in the amide substituent of lysergamides may have a profound influence on hallucinogenic activity, as reflected in the DD model. The relative potency of 5 (25% of LSD) is in agreement with clinical evaluations (see Table 1), thereby lending support to the validity of this model. It is particularly noteworthy that 17-1 represents the first amide-substituted LSD derivative with potency that surpasses the parent drug. Thus, the molecular determinants of hallucinogenic activity in the amide moiety may not be achieved optimally by the diethyl group. There has previously been no hint that this may be the case. In addition, the finding that relatively large potency differences exist for the four stereoisomers of 17 represents important evidence that the sensitivity of activity to the features of the amide substituent is not merely related to the size or physicochemical properties of this portion of the molecule. Since the four isomers of 17 differ only in the spatial arrangement of identical atoms, amide conformational properties may be of great significance in optimizing interactive forces at the receptor involved in LSD-like actions.

The complete substitution of all four stereoisomers of 17 is interesting with regard to the assessment of Hashimoto et al. (1977) referred to earlier (page 36). That is, the amide substituent may contribute to the high receptor affinity of LSD while having little effect on intrinsic activity. Thus, LSD derivatives with a variety of amide moieties may be able to produce similar activity as long as appropriate adjustments are made in the dose used. The results of the present experiment support this view but do not address the subtle qualitative differences that may become apparent in the unlikely event that these compounds are evaluated clinically.

It is, perhaps, not surprising that stereochemical properties can result in potency differences, since biological binding sites are chiral. Certainly, the results observed here are overshadowed by the "all or none" effect with respect to the correct absolute configurations at C-5 and C-8. It was somewhat unexpected, though, that such properties would result in the relatively large, 22-fold difference in potency between the most and least active stereoisomer. Assuming similar pharmacokinetics, the differences observed in this behavioral assay can probably be attributed to effects related to the drug-receptor interaction.

Stereochemical changes in portions of a drug molecule which are involved in binding interactions are known to produce the greatest effect on potency (Ariëns, 1984). The magnitude of the difference in potency between the isomers of 17 is consistent, therefore, with the idea that the amide substituent interacts with a sterically sensitive hydrophobic binding site on the receptor. The existence of such a hydrophobic region which can accommodate, in a predictable way, the substituents of the amide nitrogen has been previously suggested by Dunn and Bederka (1974). These workers also speculated that the conformational rigidity of cyclic amides may impair receptor contact.

The results of the present study suggest that if a hydrophobic bonding site is involved, stereochemical aspects of the amide alkyl groups that interact with it exert an important influence on the affinity of the interaction. The amide substituent of 17-1 may be slightly better than those of LSD and less potent compounds in optimally conforming to the requirements of such a site. This additional point of attachment may therefore be viewed as contributing significantly to the affinity of the molecule for the receptor.

Alternatively, the alkyl group(s) of the amide substituent may indirectly affect binding by influencing the conformation of another portion of the molecule. For example, the directionality of the lone pairs of electrons of the carbonyl oxygen of LSD is probably influenced by factors including the relative stability of the possible amide *N*-alkyl conformations. The various optimally stable positions adopted by the different alkyl groups in the lysergamide series may cause the carbonyl oxygen to to adopt slightly different positions in each deriviative.

It is conceivable, therefore, that the diethylamide of LSD may help to orient the lone pair electrons of the carbonyl oxygen in such a way as to optimize hydrogen bond formation with a proton of one of the amino acids which compose the receptor, thereby increasing the affinity of the drug. This possibility has been discussed previously by Kumbar (1978) in an empirically-derived conformational analysis of LSD and several other lysergamides. It was found in that study that the directionality of the carbonyl oxygen, observed in LSD, changed when the diethyl groups were substituted with other alkyl substituents. The angle of deviation from the LSD carbonyl conformation correlated with hallucinogenic potency, thus providing support for the importance of a hydrogen bonding interaction involving the oxygen (Kumbar, 1978). It may be envisioned that the isomers of 17, with their various amide conformations, differ with respect to the conformation of the carbonyl group relative to the remainder of the molecule. The resulting difference in the directionality of the nonbonded electron pairs of the oxygen may account for some degree of potency variation.

In addition, the role of the lone pair electrons of the tertiary nitrogen atom in the ergoline D-ring has been discussed extensively with respect to binding forces of the drug-receptor interaction (Chothia and Pauling, 1969; Nichols et al., 1978; Weinstein et al., 1978; Kidric et al., 1985). Variations of the amide alkyl substituent may also affect the conformation of the D-ring and the directionality of the molecular orbital of the nitrogen's lone pair electrons. In fact, the rigidity of the LSD molecule tends to increase the importance of conformational properties of the amide side chain as an influential factor in the determination of the shape of the molecule as a whole.

Whether the mechanism by which the amide alkyl groups affect the potency of lysergamides involves direct effects, indirect effects, or a combination of effects remains unknown. However, it is clear that stereochemical factors play an important role in that mechanism. Efforts directed toward the elaboration of that role are being pursued. The primary focus of most immediate concern will be to attempt to determine the absolute configurations of the isomers of 17. Other stereochemical probes of serotonin receptors involved in the actions of LSD can then be designed, synthesized, and evaluated.

In conclusion, the present study has demonstrated that certain stereochemical aspects of the amide substituent of LSD exert a significant influence on potency in producing LSD-like discriminative stimulus effects in rats. Since this behavioral paradigm seems to provide a reasonably good model for human hallucinogenic activity, a similar influence on the latter may also exist. The conformation of the amide function may directly affect binding through stereoselective interactions with a hydrophobic region on the receptor, or indirectly by inducing conformational changes elsewhere in the molecule.

Experimental Section

9,10-Didehydro-6-methyl-8ß-pyrrolidylcarboxamide-ergoline, 5. The method of Johnson et al.(1973) was successfully employed in the synthesis of 5, but superior yields were obtained using the method of Nakahara and Niwaguchi (1974). A solution of 311 mg (1 mmol) of triphenylphosphite and 68 mg (1 mmol) of imidazole in 2 mL of dry CH_3CN was added to 100 mg (0.35 mmol) of d-Lysergic acid monohydrate in 3 mL of hexamethylphosporamide (HMPA). This mixture was stirred under nitrogen at room temperature for 2 h, followed by addition of 92.3 mg (1.3 mmol) of pyrrolidine in 2 mL CH_3CN. After stirring for 8 h the reaction solution was poured into 50 mL $CHCl_3$ and washed with 3 x 40 mL of 1M NH_4OH and then extracted into 3 x 40 mL of 1% tartaric acid. The acidic solution was basified with NH_4OH and the product was extracted into 4 x 40 mL of toluene and dried over Na_2SO_4. After filtration of the drying agent and rotary vacuum evaporation of the solvent the crude product was taken up into 5 mL of 5% MeOH/$CHCl_3$ and passed through 0.5 g of Al_2O_3 in a disposable pipet in order to remove polar biproducts. The normal isomer was then separated from the iso-derivative by radial centrifugal chromatography (Chromatotron), employing a 2 mm alumina/silica plate (see Hoffman, 1987) and eluting with CH_2Cl_2 in an atmosphere of N_2 bubbled through concentrated NH_4OH and passed through a drying tube. This afforded 76.8 mg (68%) of pure 5. Recrystallization of the free base from benzene yielded a white crystalline solid: mp = 170° C (Lit. mp = 172° C, Hofmann, 1978); $[\alpha]_D$ = +26° (c=0.5, EtOH); NMR data for 5 can be found in Tables 5 and 6.

***Trans*-2,3-dimethylaziridine, 11.** To a mechanically stirred slurry of 8.2 g (216 mmol) of lithium aluminum hydride (LAH) in 200 mL of dry Et_2O, cooled in an ice bath, was added 26 g (116 mmol) of *erythro*-2-azido-3-iodobutane, 10 (Fowler et al., 1967) in 50 mL Et_2O dropwise over 45 min. The mixture was allowed to stir at room temperature for an additional 12 h, then cooled again in an ice bath and stirred vigorously while 30 mL of 10% NaOH was added dropwise over 1 h. After 45 min of additional stirring, the salts were removed by gravity filtration through filter paper and washed with an additional 100 mL of Et_2O. The combined solution was collected in an ice-cooled flask and dried over Na_2SO_4 and KOH pellets while refrigerated. In order to ensure complete drying, the solution was filtered into another flask containing fresh Na_2SO_4 and KOH every 10 h, three times. The isolation of 11 from the Et_2O was accomplished with Amberlyst-15 cation exchange resin (H form). The solution was filtered into a flask containing 28.97 g (116 mEq of acid) of dry resin and stirred, while cooling in an ice bath, for 20 min, after which the Et_2O was neutral to pH paper. The beads were filtered and transferred into a flame-dried, tared, 500 mL roundbottom flask and vacuum dried at 0.08 mm for 3 h. The beads were weighed and the difference was taken to be the weight of the product, 6.05 g (74% yield). A 1 g aliquot of dimethylaziridine-bound beads was removed for an analytical sample and to the remaining beads was added 60 mL of $CHCl_3$. The flask was cooled in an ice bath and slowly stirred while 20 mL of saturated K_2CO_3 was added in portions over 20 min. After stirring for 1 h, the supension was gravity filtered and the resin washed with 20 mL additional $CHCl_3$. The $CHCl_3$ was separated and dried as described above. The 1 g aliquot of beads was liberated in a similar fashion except $CDCl_3$ was used instead of $CHCl_3$. The reaction yield was estimated by determining the concentration of 6 in a measured aliquot of $CDCl_3$ in which the aziridine contained in 1 g of beads had been liberated. This was accomplished by comparing the integrations of the aziridine ring protons with the integration of the methylene protons from a known amount of added CH_2Cl_2. The transfer of aziridine with Amberlsyt-15 resin did not result in observable ring opening, as determined NMR. The yield of the reaction was 78%. ^{1}H-NMR ($CDCl_3$) δ 0.7-0.92 (br s, 1, NH, vanishes with D_2O), 1.19 (d, 6, CH_3, J = 5.3 Hz), 1.61-1.72 (m, 2, CH). These NMR data agree with previously reported values for this compound (Hassner et al., 1969).

***Cis*-2,3-dimethylaziridine, 14.** A procedure identical to that used for the *trans* isomer, 11, was employed except that *threo*-2-azido-3-iodobutane, 13 (Fowler et al., 1967), was used as the starting material. The yield, estimated by the method descibed for 11, was 83%. ^{1}H NMR ($CDCl_3$) δ 0.4-0.7 (br s, NH, vanishes with D_2O), 1.12 (d, 6, CH_3, J = 5.8Hz), 2.02-2.09 (m, 2, CH). These NMR data agree with previously reported values for this compound (Hassner et al., 1967).

***Erythro*-*N*-(2-chloro-1-methylpropyl)-9,10-dihydro-6-methyl-8ß-carboxamide-ergoline, (17-1 and 17-2).** Lysergic acid monohydrate (105 mg, 0.35 mmol) and 5 mL of $CHCl_3$ were placed in a flame-dried 25 mL, 3-necked roundbottom flask equipped with a condenser, an addition funnel, and a N_2 line. The stirred slurry was brought to reflux in a 90° C oil bath after which 220 mg (3.1 mmol) of *trans*-2,3-dimethylaziridine, 11, in 3 mL of $CHCl_3$ and 107 mg (0.7 mmol) of $POCl_3$ were added simultaneously, via syringe, over 2 min. The mixture was allowed to stir for an additional 5 min and was cooled to room temperature. The clear $CHCl_3$ solution was then washed with 3 x 10 mL of 1 M NH_4OH. The combined aqueous washes were extracted with 2 x 10 mL of $CHCl_3$ which was then combined and dried (Na_2SO_4) with the original $CHCl_3$ solution, while refrigerated under N_2. The drying agent was filtered and the solution was passed through a disposable pipet containing 0.5 g of Al_2O_3, eluting with 30 mL of 10% MeOH in $CHCl_3$. The solvent was then reduced in the dark by rotary evaporation with a 35° C water bath and the amber residue was taken up into 3 mL $CHCl_3$. The product was initially purified by chromatotron, as described for 5. The diastereomers of the normal isomers were then resolved using a second chromatotron with similar conditions except that a 2 mm silica plate was used and the eluting solvent was EtOAc. The solvents containing the first eluted band (17-1) and the second eluted band (17-2) were individually reduced by rotary evaporation, the residue taken up in benzene, and washed with water to remove acetamide (formed by reaction of between EtOAc and the NH_3 atmosphere), then dried over Na_2SO_4. Recrystallization of the free bases in CCl_4/hexanes yielded 22 mg of 17-1: mp = 142° C; $[\alpha]_D$ = +37° (c=0.5, EtOH), IR (neat) 1650 cm^{-1} (C=O); *m/e* 358, 360 (MH^+), 322 (MH^+ - HCl) and 19 mg of 17-2: mp = 121° C; $[\alpha]_D$ = +9.6° (c=0.5, EtOH); IR (neat) 1650 cm^{-1} (C=O); *m/e* 358, 360 (MH^+), 322 (MH^+ - HCl). High resolution mass spectrometry found the molecular weight of both 17-1 and 17-2 to be 357.1638 (calculated value = 357.1686). Combined yield = 37%. See Table 4 for tlc data, Table 5 for ^{1}H-NMR data, and Table 6 for ^{13}C-NMR data.

***Threo*-9,10-didehydro-6-methyl-8ß-*N*-(2-chloro-1-methylpropyl)-carboxamide-ergoline (17-3 and 17-4).** An identical procedure to the one used for 17-1 and

17-2 was followed up to the first chromatotron. Better resolution of the components eluting from the alumina/silica chromatotron rotor was obtained by eluting with 5% Et_2O in $CHCl_3$ instead of EtOAc. The purified products were obtained in 25% combined yield after crystallizing from CCl_4/hexanes; 17-3: mp = 158° C; $[\alpha]_D$ = +54.7° (c = 0.5, EtOH); IR (neat) 1650 cm^{-1} (C=O); *m/e* 358, 360 (MH^+), 322 (MH^+ - HCl); 17-4: mp = 115° C; $[\alpha]_D$ = +12.2° (c=0.5, EtOH); IR (neat) 1650 cm^{-1} (C=O); *m/e* 358, 360 (MH^+), 322 (MH^+ - HCl). HRMS exact mass = 357.1638 for both 17-3 and 17-4 (calculated value = 357.1686). See Table 4 for tlc data, Table 5 for ^1H-NMR data, and Table 6 for ^{13}C-NMR data.

***Erythro-N*-(2-chloro-1-methylpropyl)-benzamide, 21.** To a stirring solution of 113 mg (1.6 mmol) of 11 and 126 mg (1.6 mmol) of pyridine in 1 mL of dry Et_2O, cooled in an ice bath and under nitrogen, was added 225 mg (1.6 mmol) of 18 in 2 ml of Et_2O dropwise over 5 min. The ice bath was removed and the reaction mixture was allowed to stir at room temperature for 4 h. The solution was then diluted with 15 mL of additional Et_2O and washed with 2 x 20 mL 3N HCl, 2 x 20 mL 1N NaOH, 2 x 30 mL of saturated NaCl, and dried over $MgSO_4$. The drying agent was filtered and the ether was rotary evaporated. Recrystallization of the product from hexanes afforded 155 mg (54%) of 21 as a white, fluffy solid, mp = 114-115° C. ^1H-NMR ($CDCl_3$) δ 1.29 (d, 3, CH_3, J = 6.6 Hz), 1.55 (d, 3, CH_3, J = 6.6 Hz), 4.33-4.50 (m, 2, CH), 6.34 (br s, 1, NH), 7.71-7.84 (m, 2, ArH). ^{13}C-NMR ($CDCl_3$) δ 167.24 (C=O), 131.63, 128.62, 126.93 (ArC), 62.77 C-Cl), 49.66 (C-NH), 22.02 (Cl-C-CH_3), 13.67 (NH-C-CH_3). IR ($CHCl_3$) 3340 (NH), 1635 (C=O), 1530 (NH) cm^{-1}. *m/e* 212, 214 (MH^+), 176 (MH^+ - HCl), 148 (MH^+ - C_2H_5Cl), 105 (MH^+ - C_4H_9Cl).

***Threo-N*-(2-chloro-1-methylpropyl)-benzamide, 21.** A procedure identical to that used for *erythro*-21 was employed except that 14 was used intead of 11. The crystallized yield was 43%, mp = 94-95° C; ^1H-NMR ($CDCl_3$) δ 1.36 (d, 3, CH_3, J

= 6.7 Hz), 1.56 (d, 3, CH_3, J = 6.7 Hz), 4.19 (q of d, 1, J = 6.7, 2.1 Hz, CH), 4.42-4.57 (m, 1, CH), 6.19 (br d, J = 8.5 Hz, 1, NH), 7.40-7.47 (m, 3, ArH), 7.73-7.78 (m, 2, ArH); ^{13}C-NMR ($CDCl_3$) δ 167.27 (C=O), 131.44, 128.59, 127.14 (ArC), 67.58 (C-Cl), 49.39 (C-NH), 22.99 (Cl-C-CH_3), 19.96 (NH-C-CH_3). IR ($CHCl_3$) 3330 (NH), 1630 (C=O), 1520 (NH) cm^{-1}. *m/e* 212, 214 (MH^+), 176 (MH^+ - HCl), 148 (MH^+ - C_2H_5Cl), 122 (MH^+ - C_4H_7Cl).

***Threo*-3-amino-2-butanol, 23.** To a stirring solution of 3.21 g (30 mmol) of benzylamine in 75 mL CH_2Cl_2, under a nitrogen atmosphere, was added 15.72 mL of a 1.9 M (29.88 mmol) solution triethylaluminum, dropwise over 5 min. After stirring for an additional 30 min, 2.16 g (30 mmol) of 22 was added and the mixture stirred at room temperature for 14 h. After the dropwise addition of 30 mL 5M NaOH over 45 min, the mixture stirred for 2 h. The organic layer was washed with 2 x 100 mL H_2O, 3 x 100 mL of saturated NaCl, and dried over $MgSO_4$. The drying agent was filtered and the solvent reduced *in vacuo*. The residue was taken up in 50 mL of 2-propanol, acidified with HCl and recrystallized with the addition of ether, affording 5.82 g (90%) of *threo*-3-(benzylamino)-2-butanol, mp = 132-134° C. Debenzylation of 4.5 g (20.88 mmol) of this amine in 300 mL of methanol with 300 mg of 10% Pd/C was accomplished by shaking under 50 psi H_2 for 36 h. The catalyst was filtered and the methanol was rotary vacuum evaporated. The residue was dissolved in 50 mL CH_2Cl_2 and basified with NH_4OH, resulting in the formation of a thick white precipitate. The filtered solution was dried over Na_2SO_4, filtered, and reduced *in vacuo* yielding 1.45 g (78%) of 23 as an oil: ^{1}H-NMR ($CDCl_3$) δ 3.35-3.42 (m, 1 , CH), 2.65-2.72 (m, 1, CH), 2.49 (br s, 3, NH_2 and OH), 1.17 (d, J = 6.2 Hz, 3, CH_3), 1.11 (d, J = 6.4 Hz, 3, CH_3).

***Threo*-*N*-(2-chloro-1-methylpropyl)-benzamide, 21 (alternate synthesis).** 1 g (11.24 mmol) of 23 and 1.13 g (11.2 mmol) of triethylamine were stirred in 30

Threo-N-(2-chloro-1-methylpropyl)-benzamide, 21 (alternate synthesis). 1 g (11.24 mmol) of 23 and 1.13 g (11.2 mmol) of triethylamine were stirred in 30 mL CH_2Cl_2, under nitrogen, cooled in an ice bath. After dropwise addition of 18 over 10 min, the reaction mixture was stirred for 10 min while triethylamine hydrochloride formed as a thick white precipitate. This salt was filtered and the filtrate was washed with 3 x 50 mL 5% $NaHCO_3$, 3 x 50 ml of 1N HCl, 50 mL saturated NaCl, and dried was over Na_2SO_4. After filtration and rotary vacuum evaporation of the solvent, the product, *threo-N*-(2-hydroxy-1-methylpropyl)-benzamide, was purified by centrifugal chromatography using a 4 mm silica plate and eluting with CH_2Cl_2: 1.49 g (69%), R_f = 0.24 in 3:1 EtOAc/hexanes. Treatment of 1 g (5.18 mmol) of this alcohol with 1.29 g (5.7 mmol) of triphenylphosphine in 50 mL CCl_4, after refluxing on a steam bath for 6 h, resulted in the formation of the product, *threo*-21. Purification was accomplished by rotary evaporation of the CCl_4 and centrifugal chromatography on a 2 mm silica rotor, eluting with 5% Et_2O in CH_2Cl_2. Recrystallization from hexanes afforded 540 mg (50%) of *threo*-21. The mp, IR and NMR data for this product and that prepared by the first method were identical.

Table 4. Tlc R_f values for lysergic acid amides.

	SOLVENT SYSTEM[a]		
COMPOUND	10% $Et_2O/CHCl_3$[b]	EtOAc[b]	$CHCl_3$[c]
1	0.30	0.32	0.61
iso-1	0.15	0.27	0.46
17-1	0.26	0.42	0.53
17-2	0.21	0.31	0.52
17-3	0.26	0.40	0.53
17-4	0.24	0.34	0.54

[a] 5 drops of NH_4OH added to all solvents.
[b] Silica tlc plate.
[c] Alumina tlc plate.

Table 5. ^{1}H-NMR data for lysergamides.[a]

PROTON	1	5	17-1	17-2	17-3	17-4
1	7.94bs	8.04bs	8.03bs	8.07bs	7.98bs	8.07bs
12-14	7.13-7.22m	7.14-7.37m	7.15-7.24m	7.13-7.24m	7.11-7.26m	7.11-7.27m
2	6.90s	6.90s	6.92-6.93m	6.92-6.93m	6.92-6.93m	6.92-6.93m
9	6.34s	6.40s	6.43-6.45m	6.43-6.45m	6.46 6.48m	6.43-6.45m
8	3.65-3.80m	3.79-3.85m	3.53-3.61m	3.63-3.66m	3.61-3.73m	3.62-3.71m
4β	3.55dd	≈3.6 (buried)	3.37-3.44m	3.36-3.40m	3.42-3.50m	3.37-3.42m
5	3.19-3.25m	3.25-3.27m	3.30-3.36m	3.29-3.33m	3.34-3.37m	3.30-3.35m
7α	3.01-3.07m	3.05-3.22m	3.05-3.13m	3.03-3.06m	3.04-3.12m	3.02-3.10m
7β	2.89t	2.88t	2.63-2.76m	2.78-2.82m	2.74-2.84m	2.74-2.84m
4α	2.62-2.69m	2.68-2.74m	2.67-2.82m	2.76-2.81m	2.71-2.79m	2.71-2.82m
N-CH_3	2.59s	2.59s	2.60s	2.63s	2.61s	2.64s
Amide Protons:						
NH	-	-	7.44m	7.60m	7.71m	7.53m
α	3.39-3.49m	3.44-3.58m	4.23m	4.20m	4.28m	4.30m
β	1.18, 1.24m	1.82-2.08m	4.25m	4.34m	4.13m	4.13m
N-CH-C$\underline{H}_3$			1.20d	1.17d	1.25d	1.23d
Cl-CH-C$\underline{H}_3$			1.48d	1.50d	1.46d	1.51d

[a] All ^{1}H-NMR data were obtained on a Chemagnetics A-200 instrument using $CDCl_3$ as a solvent and TMS an internal reference.

s =	singlet	d =	doublet	t =	triplet
bs =	broad singlet	dd =	doublet of doublets	m =	multiplet

Table 6. ^{13}C-NMR data for lysergamides.[a]

CARBON	1	5	17-1	17-2	17-3	17-4
2	118.20	118.20	118.66	118.74	118.55	118.74
3	109.55	109.55	109.78	109.79	109.74	109.76
4	27.42	27.45	24.14	23.45	23.69	23.33
5	63.22	63.17	62.41	62.44	63.09	62.08
7	56.05	55.33	52.13	51.11	52.40	51.11
8	41.98	42.14	42.15	42.09	41.90	42.11
9	119.57	119.09	118.95	119.25	119.38	119.28
10	136.19	136.25	138.21	138.21	138.17	138.37
11	128.08	128.30	129.11	128.30	129.40	129.48
12	112.51	112.59	111.97	111.78	111.91	111.81
13	123.19	123.25	123.27	123.22	123.38	123.28
14	110.73	110.81	110.97	111.03	110.09	111.13
15	133.91	133.94	133.87	133.88	133.86	133.85
16	126.12	126.15	126.25	126.28	126.07	126.28
C=O	171.64	171.00	172.60	172.83	173.11	173.29
N-CH$_3$	43.89	43.91	44.42	44.42	44.48	44.61
α-C[b]	40.23	46.73	49.06	49.12	48.88	48.97
	39.97	46.04	21.87	21.76	22.52	22.63
ß-C[c]	14.88	26.30	60.97	60.51	60.51	60.42
	13.14	24.28	13.96	13.57	19.45	19.24

[a] All proton-decoupled ^{13}C-NMR data were obtained on a Chemagnetics A-200 instrument using CDCl$_3$ (solvent and internal reference).

[b] The isomers of 17 have only one α-carbon; the ß-CH$_3$ is also listed.

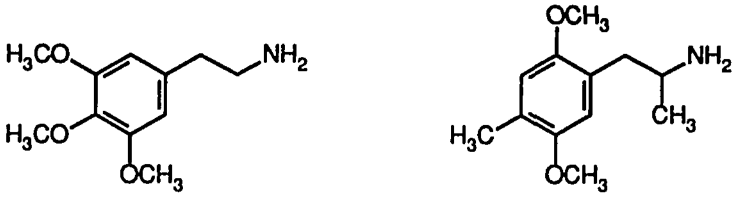
[c] For the isomers of 17, the first value is assigned to the ß-carbon bonded to the Cl; the second value is the terminal CH$_3$.

CHAPTER 3

THE 4-ALKYL SUBSTITUENT IN HALLUCINOGENIC AMPHETAMINES

Introduction

Our understanding of ß-phenethylamine derivatives as hallucinogenic agents began in 1896 when Heffter identified mescaline as the active component of the peyote cactus (Heffter, 1896). This was followed by the determination of the structure (3,4,5-trimethoxyphenethylamine, Figure 20) and the synthesis of mescaline by Späth (1919). From an historical perspective, mescaline is one of the more important of the "classical hallucinogens", despite the fact that its potency has been exceeded, by nearly three orders of magnitude, by the potencies of several analogues (Nichols, 1986a). However, in pharmacological investigations, 2,5-dimethoxy-4-methylphenylisopropylamine (DOM, Figure 20), has been the prototype for this drug class.



MESCALINE DOM

Figure 20. Molecular structures of mescaline and DOM.

A large number of mescaline derivatives have now been produced as tools for investigations of structure-activity relationships (SARs). These efforts were facilitated by the relative simplicity of these molecules and the availability of a wide variety of related aromatic ring-substituted starting materials. The numerous SAR studies of hallucinogenic compounds related to mescaline have been well reviewed (see Shulgin, 1978; Shulgin 1981; Nichols, 1981; Nichols and Glennon, 1984; Nichols, 1986a) and have focused on the alkylation of the side chain, alkylation of the amino group, and the orientation and character of the aromatic ring substituents (Nichols, 1986a).

For example, it was noted that activity could be optimized by rearranging the three methoxy groups from the 3,4,5-orientation found in mescaline, to a 2,4,5-orientation, and by adding an α-methyl group, as in TMA-2 (Figure 21).

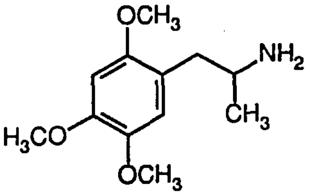
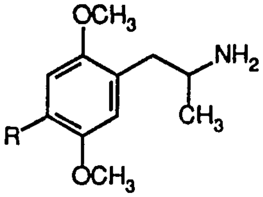


Figure 21. Molecular structure of TMA-2.

A majority of the subsequent studies involved α-methylphenethylamine derivatives, also referred to as phenylisopropylamines or "amphetamines" (Shulgin, 1966; Shulgin 1976). A further increase in potency was observed (see references cited in Shulgin and Dyer, 1975) when the 4-methoxy of TMA-2 was replaced with a methyl group, resulting in DOM (Figure 20). This compound served as a starting point for the study of hallucinogenic activity in a homologous series of 4-alkyl-2,5-dimethoxyphenylisopropylamines (Shulgin and Dyer, 1975). The names, or codes, for these compounds begin with the letters "DO" indicating "desoxy" (loss of the 4-oxygen of TMA-2), followed by an abbreviation for the 4-substituent (Shulgin, 1978). The results of this study, shown in Table 7, led to the conclusion that the 4-alkyl substituent had a critical influence on the hallucinogenic activity in this family of compounds.

Table 7. Hallucinogenic potency of 4-alkyl-2,5-dimethoxyamphetamines.[a]



Code	R	Hallucinogenic Potency[b]
2,5-DMA	H	8
DOM	CH_3	80
DOET	CH_2CH_3	100
DOPR	$CH_2CH_2CH_3$	80
DOBU	$CH_2CH_2CH_2CH_3$	36
DOAM	$CH_2CH_2CH_2CH_2CH_3$	10
DOTB	$C(CH_3)_3$	-[c]

[a] Data from Shulgin and Dyer (1975).

[b] expressed in terms of mescaline units, derived by dividing the effective human dose of mescaline by that of the compound.

[c] this compound has not been established as hallucinogenic.

Within this homologous series, optimum activity is associated with a straight chain alkyl group, two and three carbons in length (Shulgin and Dyer, 1975). The potency increases by an order of magnitude as the 4-hydrogen of 2,5-DMA is replaced by a short alkyl chain, then decreases if the chain length exceeds four carbons. In addition, the lack of hallucinogenic activity for the tertiary butyl derivative, DOTB, suggested that branching of the 4-alkyl substituent was not tolerated.

Similar results were obtained in animal studies. Aldous et al. (1974) found that the 4-substituent was essential for high activity, as measured in the rabbit hyperthermia model. The potency of 2,5-DMA increased by an order of magnitude with the addition of the 4-methyl substituent in DOM. Activity remained high for DOET and DOPR, but decreased dramatically when branching, in the form of a *t*-butyl, or an *i*-propyl group, was introduced at this position.

Drug discrimination studies provided further evidence for the deleterious effect of excessive chain length or branching on activity. In rats trained to discriminate DOM from saline (Glennon et al., 1982b), it was found that DOET, DOPR and DOBU were all perceived as similar to the training drug, and were approximately similar to one another in potency. The addition of one methylene to the 4-butyl substituent of DOBU, as in DOAM, or rearrangement to a *t*-butyl, as in DOTB, resulted in the loss of DOM-like DS properties.

Interestingly, it has been noted that the discriminative stimulus properties of DOM, and those of the indole-based hallucinogen 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), were substantially similar and might share a common mechanism of action (Young et al., 1981; Glennon et al., 1986). In a study employing 5-MeO-DMT as the training drug in rats, DOTB and DOAM were distinguishable from this hallucinogen, while 2,5-DMA and DOM were not (Glennon et al., 1981). This was consistent with the studies described above. Surprisingly, however, stimulus generalization was not observed for DOET, DOPR, and DOBU (Glennon et al., 1981a). An explanation for the difference between the DS properties of these analogues, relative to those of DOM and 5-MeO-DMT is not yet apparent.

In humans, at effective threshold levels, DOM and DOET seem to produce similar effects, although the qualitative aspects of the intoxication produced by the longer alkyl chain analogues have not been adequately investigated (Shulgin, 1978). However, the majority of experimental data provide strong evidence that DOM, DOET, and DOPR produce similar effects with comparable potency. Hallucinogenic activity is attenuated when the 4-alkyl substituent is extended from three to four to five carbons in length, and apparently is abolished when branching is introduced. This empirically derived relationship is not well understood on theoretical grounds.

Gupta et al. (1983) have concluded that "the 4-substituent has been found to be of unique importance" in hallucinogenic substituted amphetamines. The possible reasons for this have been discussed by Nichols (1986a), and include an enhancement of general lipophilicity (leading to higher drug levels in the brain), the prolongation of the duration of action by retarding oxidative metabolism at the 4-position, and enhancement of receptor affinity. Although the question of how the 4-substituent affects drug action is still unclear, its influence on the drug-receptor interaction is probably important. The net effect of a particular chemical group, at this critical position of the drug molecule, probably depends on a combination of factors.

The observed correlation of hallucinogenic activity with log P, for phenethylamine derivatives, led to the emphasis of the importance of distribution and transport of the drug to the receptor (Barfknecht et al., 1975). However, the log P values for the straight-chain and branched-chain analogues in the 4-alkyl 2,5-DMA series are similar. Thus, the deleterious effect of branching would not be expected to be due to differences in the abilities of these compounds to reach the receptor. Although this possibility cannot be ruled out, other factors are probably more critical.

The possibility that metabolic activation is actually critical to the mechanism of action of hallucinogens has been discussed (Anderson et al., 1978), but the importance of such processes has yet to be demonstrated (Nichols and Glennon, 1984). The relationship between metabolic factors and hallucinogenic potency was investigated for the positional isomers of trimethoxyamphetamine (Mitoma, 1970), but not for the series of 4-alkyl analogues of 2,5-DMA. It has been noted, however, that the oxidation of the 4-alkyl group proceeds more slowly for DOET relative to DOM, and that this could account for the slightly greater potency of the former (Castagnoli, 1978).

While the increased activity of 2,5-DMA, upon 4-substitution, may relate to a blocked metabolic pathway, the broad range of potencies observed for the various 4-alkyl derivatives (Table 7) would not be expected to be primarily attributable to metabolic differences (Glennon et al., 1982b). Therefore, in the absence of evidence to the contrary, it does not seem likely that differential metabolism plays a dominant role in determining the potency of compounds in this series.

The effects of the 4-substituent on factors which influence the drug-receptor interaction are probably more critical to hallucinogenic activity than pharmacokinetic effects. The receptor affinity of hallucinogenic amphetamines, in general, is thought to depend on a combination of electronic, hydrophobic, and steric factors (references cited in Gupta, 1983). However, as reflected in the lack of reliable quantitative models, these factors are not presently well understood.

It is generally thought that at least two pharmacophoric sites, the charged ammonium group and the aryl moiety, serve as the foci of attractive forces between the hallucinogen molecule and the receptor (Anderson, et al., 1978 and references cited therein). Consideration of the possible binding forces between the receptor and the aryl moiety has led to speculation that the formation of a charge-transfer (electron donor-acceptor) complex may be critical. There has been considerable interest for many years in charge-transfer processes within biological systems (Szent-Gyorgyi, 1960).

This idea was first suggested with respect to hallucinogenic drug action by Karreman et al. (1959), based on the electron-donor capabilities of LSD. Subsequently, molecular orbital calculations of active hallucinogens and their inactive analogues led to the proposal of an electron-donation model of the drug-receptor interaction (Snyder and Merrill, 1965). In the years that followed, the involvement of charge-transfer processes has received support from numerous theoretical and experimental studies (see Nichols, 1981).

The term "charge-transfer complex" is used to describe interactions between molecules (not conveniently described in terms of dipole-dipole, dipole-induced dipole, or van der Waals interactions) resulting in complexes which may exhibit spectral absorptions and crystal structures other than those of the original components, produce a color upon formation, but which reversibly dissociate (Doukas, 1975). The details of the electronic characteristics of these complexes in general (Mulliken and Person, 1969), and for those formed between aromatic systems (Suppan, 1969), have been described.

In aromatic systems, the term "π complex" is used, since these interactions are associated with the electrons of the easily perturbed π framework (Doukis, 1975). The electron-donor molecule may be an aromatic system substituted with electron donating groups, while electron withdrawing groups may characterize an electron-acceptor molecule. Thus, the 2,5-dimethoxy substituents lead to increased electron density in the benzene π-cloud, making the series of compounds under discussion suitable donors. The electron-acceptor species would presumably be associated with the binding site or receptor.

The electron transfer from the hallucinogen to the receptor is considered to be an outer-sphere, charge transfer process (Kolb, 1987). In the stabilization of the drug-receptor interaction, a complete transfer of an electron from the donor to the acceptor need not occur. Rather, a more likely situation would involve a mutual perturbation of the respective π clouds that would stabilize the molecular association (Doukas, 1975). The charge redistribution which accompanies complex formation could enhance electrostatic interactions without an actual transfer of charge (Weinstein et al., 1978).

Sung and Parker (1972) employed NMR measurements to calculate the association constants of molecular complexes formed between several active methoxyamphetamines and a model electron-deficient receptor (1,4-dinitrobenzene). These workers found a good correlation between complex formation and hallucinogenic potency. However, steric factors were not directly studied, as molecules were selected which fulfilled a preset, empirically-derived steric requirement. The ability of interacting molecules to orient themselves so as to achieve maximum orbital overlap is of major importance and introduces the necessity of mutual steric accomodation (Doukas, 1975). Indeed, inhibition of complex formation was observed in model systems where bulky groups were attached to donor molecules (Charton, 1966). In addition, the receptor that interacts with hallucinogens has been proposed to contain a planar surface which does not tolerate steric bulk projecting from the binding face of the drug (Nichols, 1981) and data exist which support this idea (Nichols and Glennon, 1984).

Thus, steric factors, possibly related to the formation of a charge-transfer complex between hallucinogenic amphetamines and their binding sites, may be important in understanding the attenuation of activity resulting from branching in the 4-alkyl substituent. The experiments described in this chapter were designed to investigate the effects of steric bulk on the LSD-like DS properties of 4-alkyl-2,5-DMA analogues. The position of alkyl branching, relative to the aromatic ring, and stereochemical aspects of the added bulk were studied.

Rationale for the Selection of Target Compounds

If the electron-rich benzene ring of the amphetamine derivative interacts with the receptor in a close, planar fashion, the steric effect of branching in the 4-alkyl substituent might be expected to impair the interaction less when positioned distal to the ring. This hypothesis was tested by synthesizing and evaluating two isomers of 4-butyl-2,5-dimethoxyamphetamine. As illustrated in Figure 22, the 4-substituent in 24 is an isobutyl group while 25 is substituted with a secbutyl group. These compounds can be viewed as analogues of the potent derivative DOPR, in which a methyl branch is added to either the ß-carbon of the alkyl group, as in 24, or to the α-carbon, as in 25.

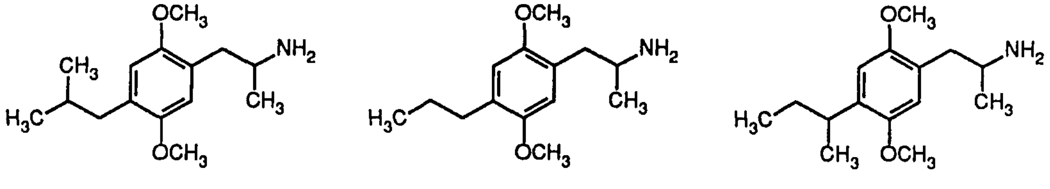

24 DOPR 25

Figure 22. Molecular structures of DOPR and the proposed derivatives.

By comparing these novel analogues, the importance of steric effects on activity may be more evident. Except for the isomeric relationship of the 4-butyl substituent, they would be expected to have similar pharmacokinetic, electronic, and quantum-chemical properties. Thus, from the above reasoning, one might predict that branching which is removed from the aromatic ring by one methylene, as in 24, should be associated with greater potency compared to 25, where the methyl branch is adjacent to the ring.

In the initial stage of this project, compounds 24 and 25 were prepared without regard to stereochemistry (Oberlender et al., 1984). The carbon α-to the nitrogen is chiral and it is well established that the *R*-(-)-isomers of hallucinogenic amphetamines elicit activity with potency that is 4-10 fold greater than that of the *S*-(+)-isomers (Nichols and Glennon, 1984). It was decided that racemic mixtures would be evaluted first, and the synthesis of optically pure isomers could be considered, if needed, later.

However, in 25, a chiral center that is more critical to the present investigation is introduced with the addition of the methyl branch on the 4-substituent alkyl carbon, α- to the ring. If the spatial orientation of this chiral center is critical, with respect to the drug-receptor interaction, a difference in potency might be observed between the isomers of 25, in which the *sec*-butyl group exists in the *R* (*R*-25) or *S* (*S*-25) absolute configurations.

A difference in the conformational properties of these compounds, as they interact with the receptor surface, was expected based on a molecular mechanics analysis previously carried out in a model system (Nichols and Weintraub, 1982). In that study, the enantiomers of 2-(2-butyl)anisole were used to model *R*- and *S*-25. A phenyl ring, placed in a parallel plane 3.3 Å from the anisole ring, was used to model a flat receptor surface, as illustrated below for the *R*-isomer.

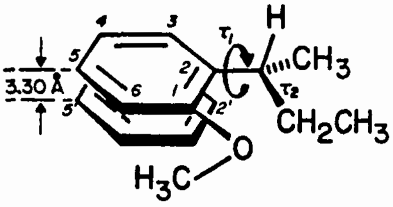


Figure 23. Model system of Nichols and Weintraub for the conformational analysis of the receptor binding interaction of *R*-25.

This simplified system allowed a comparison of the steric factors in the interaction of the *R* and *S* 2-butyl substituents with a chiral environment, as might occur upon receptor binding. Differences in quantum chemical properties were assumed to be minimal and ignored. The deletions of the side chain and the 2-methoxy group were justified on the basis that their contributions could be assumed to be identical and would not perturb the 2-butyl group's conformational equilibrium.

The results of this analysis demonstrated a clear difference between the conformational properties of the two enantiomers as studied in this chiral environment (Nichols and Weintraub, 1982). It was also found that on the basis of probability, *R*-25 can best approach the receptor surface from its α-face (that which is facing the benzene ring in Figure 23), while *S*-25 can best approach it from its β-face. Thus, in addition to gaining an understanding of stereochemical aspects of branching in the 4-substituent, the synthesis and evaluation of these compounds were anticipated to provide data that would be useful in testing predictions of the orientation of substituted amphetamines in the drug-receptor interaction (Weintraub and Nichols, 1978).

Synthetic Chemistry Discussion

The synthesis of 24 has been described previously (Oberlender et al., 1984) and is included here for the sake of completeness. It was accomplished in eight steps as outlined in Figure 24. Friedel-Crafts acylation of p-dimethoxybenzene 26, with isobutyryl chloride 27, yielded the ketone 28, which was then reduced with sodium borohydride to the alcohol 29. Dehydration with the sulfonic acid cation-exchange resin Amberlyst-15, yielded the olefin 30, which was then catalytically hydrogenated to 31. The aldehyde 32 was formed by the reaction of 31 with dichloromethylmethyl ether, followed by aqueous workup. Condensation of 32 with nitroethane yielded the nitropropene derivative 33, which was reduced with lithium aluminum hydride to the amine 24. Acidification with HCl yielded the salt that was used in the DD assay.

Racemic 25 was first prepared as described previously (Oberlender et al., 1984) by a similar method. Although 25 is a mixture of diastereomers, they did not resolve on TLC, nor did repeated recrystallizations of the hydrochloride salt alter the melting point of this mixture. Several attempts

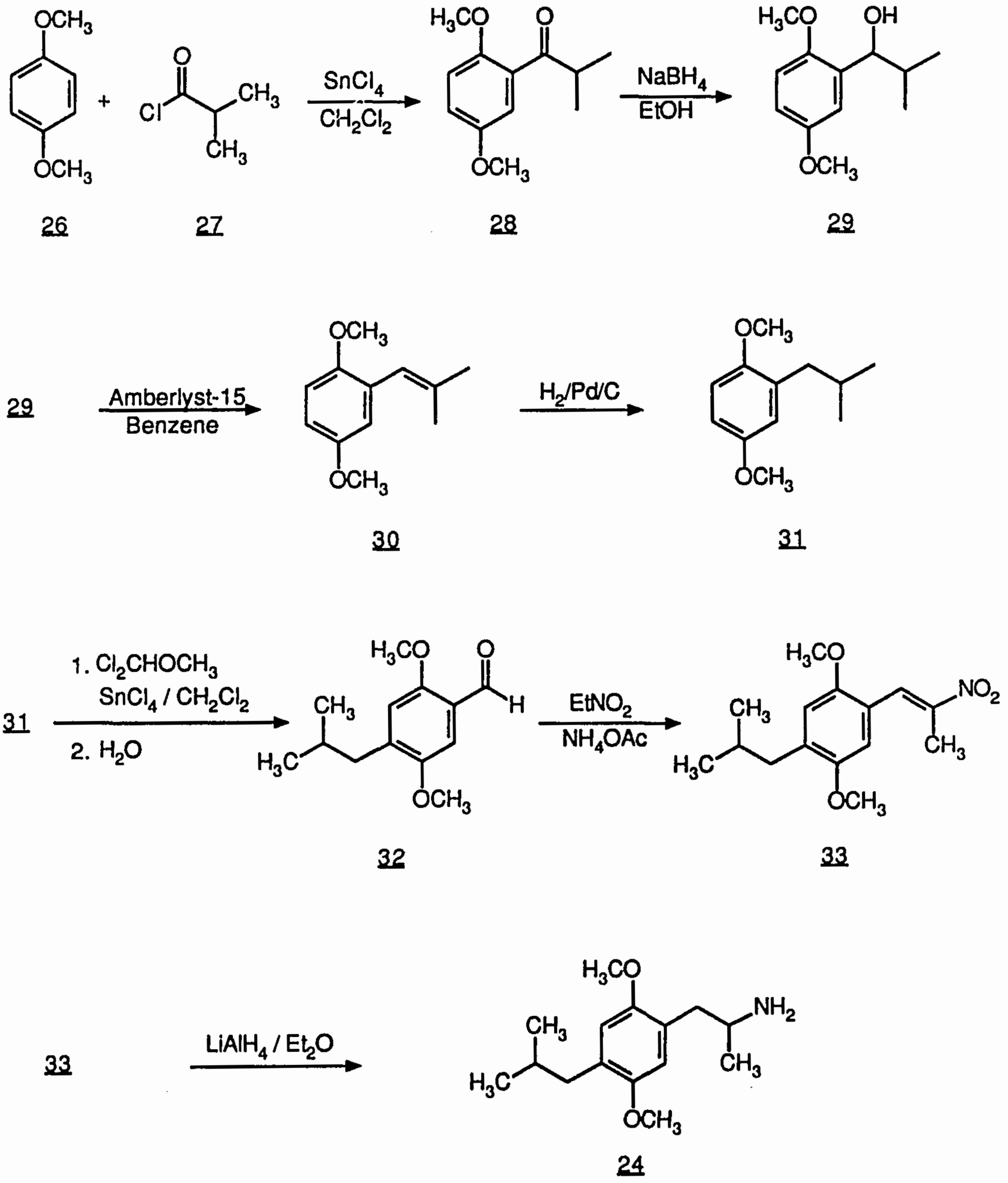
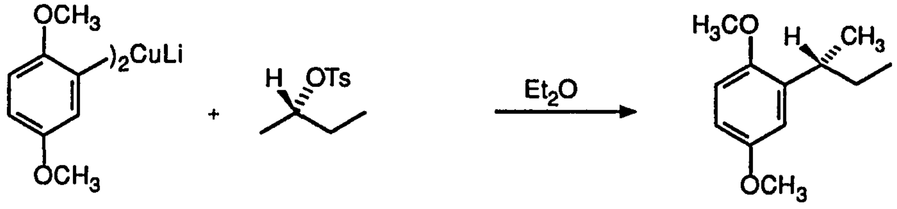


Figure 24. Synthesis of 4-(2-methylpropyl)-2,5-dimethoxyamphetamine.

to prepare the individual isomers of 25 through the chemical resolution of various intermediates had previously proven unsuccessful. Effective asymmetric syntheses were therefore studied and several possibilities were considered. The key intermediates of these syntheses were identified as the 2*R* and 2*S* isomers of 2-(2,5-dimethoxyphenyl)-butane, 36. The use of asymmetric hydrogenation catalysts on the prochiral olefin was rejected because of the poor optical purity that could be expected for the product (Knowles, 1983).

Three routes were identified that could lead to the preparation of the optically pure enantiomers of 36 (Figures 25-27). The first route was based on the work of Johnson and Dutra (1973), who found that the reaction of *S*-(+)-2-butyl tosylate, or mesylate, with lithium diphenylcuprate in ether afforded *R*-(-)-2-phenylbutane. The optical purity of the product indicated that the reactions had proceeded with 100% inversion of configuration. This procedure, shown in Figure 25, employing lithium di-(2,5-dimethoxyphenyl)cuprate 34 and *S*-(+)-2-butyl tosylate 35, yielded the desired product, 36.

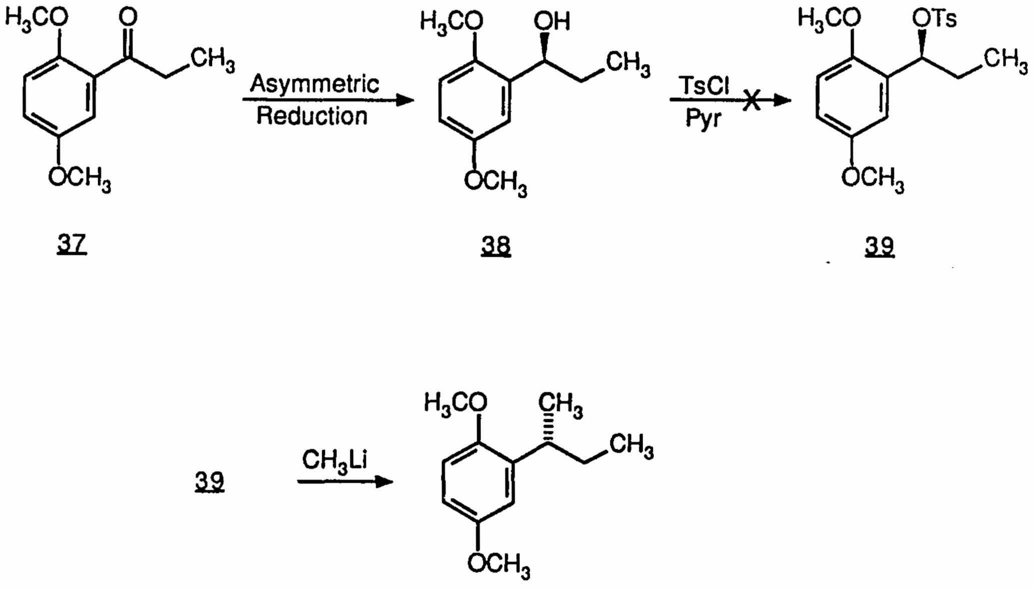


34 *S*-(+)-35 *R*-(-)-36

Figure 25. Synthesis of *R*-(-)-2-(2,5-dimethoxyphenyl)butane by the method of Johnson and Dutra.

Since the commercially available 2-butanol (Aldrich) was not optically pure (82% ee), and the separation of the product from unreacted dimethoxybenzene was tedious, other methods were examined. However, the above procedure was valuable in the confirmation of absolute configuration.

The second route that was considered attempted to introduce chirality at the benzylic carbon with an asymmetric reduction of 1-(2,5-dimethoxyphenyl)-propanone 37. It was anticipated that the alcohol 38, could then be converted to the tosylate 39. Subsequent displacement in an S_N2 reaction with methyl lithium would afford the enantiomers of 36. This route is shown in Figure 26.



R-(-)-36

Figure 26. Attempted synthesis of *R*-(-)-2-(2,5-Dimethoxyphenyl)-butane from a benzylic ketone.

Although the chiral alcohol 38 (96% ee) was successfully synthesized using the isomers of diisopinocampheylchloroborane (Brown et al., 1988), careful attempts to convert the racemic alcohol to the tosylate or mesylate consistently resulted in elimination to the olefin. The isomers of 2-(2,5-dimethoxyphenyl)butane were successfully prepared, as shown in Figure 27, by using an indirect method to accomplish an asymmetric hydrogenation of *Z*-2-(2,5-dimethoxypheny)-2-butene, 42.

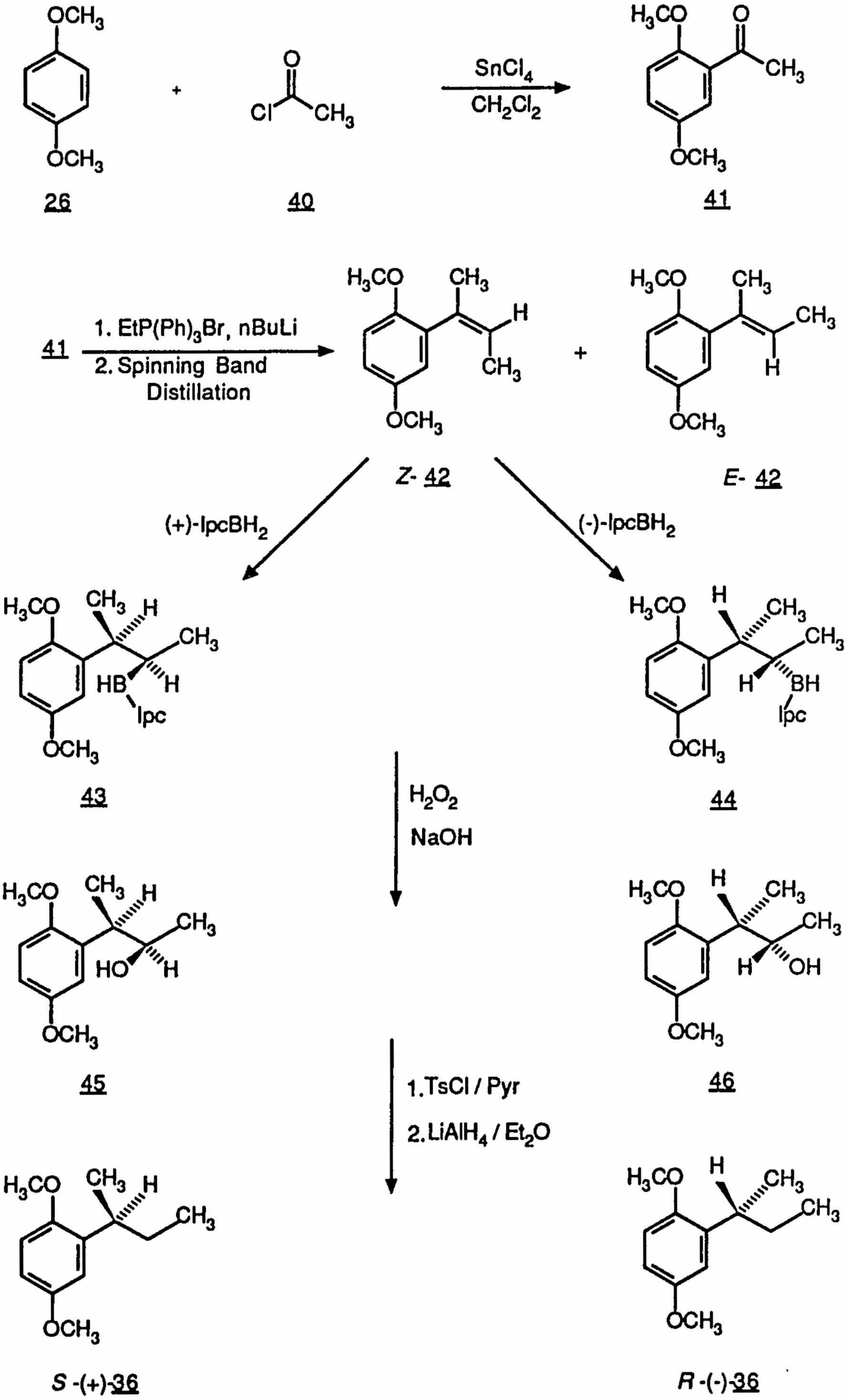


Figure 27. Asymmetric synthesis of *R*-(-)- and *S*-(+)-2-(2,5-dimethoxyphenyl)butane *via* hydroboration/oxidation.

The chiral benzylic center was formed by the hydroboration of the olefin with (+)- or (-)-monoisopinocampheylborane (IpcBH$_2$). Brown and coworkers (Mandal et al., 1980; Brown et al., 1982a) had previously demonstrated the synthetic utility of IpcBH$_2$ for phenyl-substituted tertiary olefins. Optical purities greater than 80% were reported for the hydroboration of 2-phenylbutene. In addition, a subsequently published report (Brown and Singaram, 1984) described the possibility of improving enantiomeric excess of the product by simply recrystallizing the intermediate isopinocampheylalkylborane. Thus, the isopinocampheyl group is used for both optical induction and for upgrading the dialkylborane products of lower optical purity.

The synthesis started with the Friedel-Crafts acylation of p-dimethoxybenzene 26 with acetyl chloride, to form the ketone 41. The Wittig reaction of 41 with ethyltriphenylphosphorane (Schlosser et al., 1986) afforded a mixture of 70% *Z*- and 30% *E*-olefins, 42, which were separated by spinning band distillation. The *Z*-olefin was then reacted with optically pure (+)- or (-)-IpcBH$_2$ (Brown et al., 1982b), yielding (2*R*,3*S*)- and (2*S*,3*R*)-3-(2,5-dimethoxyphenyl)-2-butylboranes, 43 and 44, respectively.

Initial attempts to improve the optical purity of the products of the reactions of *E*-42 with (+)- and (-)-IpcBH$_2$ were unsuccessful, since the intermediate dialkylboranes could not be induced to crystallize. When *Z*-42 was used, products 43 and 44 were solid, but crystallization of these intermediates did not improve optical purity (as determined by the method described in the experimental section for 45). However, the 82-83% ee that was achieved in these reactions meant that no more than 9% of the minor isomer was present in the mixture. This was felt to be suitable for the determination of pharmacological differences.

Attempts to replace the IpcBH group with hydrogen through protonolysis (Brown and Murray, 1959) with acetic, propionic, or isobutyric acid were unsuccessful, leading to decomposition of product. Instead, 43 and 44 were filtered and converted by hydrogen peroxide oxidation to the (2*R*,3*R*)- and (2*S*,3*S*)-alcohols, 45 and 46 respectively. Conversion of the alcohols to the tosylates was followed by reduction with lithium aluminum hydride, affording *R*-(-)-36 and *S*-(+)-36. The absolute configurations were confirmed by comparison of the sign of rotation of the products to material of unambiguous stereochemistry prepared by the previously described coupling reaction (Figure 25).

The synthesis then proceeded with the formylation of the isomers of 36 using dichloromethylmethyl ether. Initially, it was not clear whether the presence of tin (IV) chloride and free HCl in the reaction mixture would lead to racemization. To confirm that this did not occur, a sample of *R*-(-)-36, of known optical purity, was converted to the aldehyde by three different methods and the rotations were compared for the products obtained.

The first method was the one just described. The second was the milder Vilsmeier-Haack reaction using *N*-methyl formanilide and phosphorous oxychloride. The third method was that of Weyerstahl et al. (1970), by which 2-phenylbutane was iodinated, lithiated through a lithium-halogen exchange reaction, and then formylated by quenching with DMF, without loss of optical activity. All three methods resulted in aldehyde of comparable rotation (within 0.5°), thereby demonstrating the lack of racemization with the more convenient first procedure. Completion of the synthesis was accomplished in a manner similar to that previously described for 24, illustrated in Figure 28 for *S*-(+)-25. The same procedure was used for the *R*-(-)-isomer.

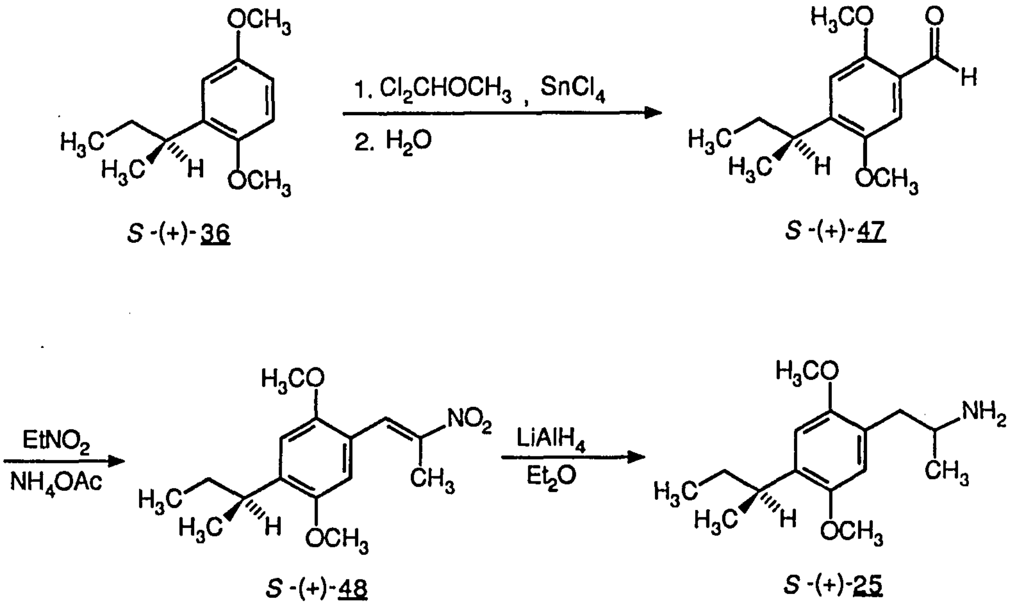


Figure 28. Synthesis of the sidechain of *S*-(+)-25.

Evaluation of LSD-like Discriminative Stimulus Properties

The results of substitution testing in rats trained to discriminate LSD from saline are presented in Table 8 and Figure 29. The test compounds are listed in Table 8 in order of decreasing potency and graphically represented in Figure 29, where the thick horizontal lines represent the 95% confidence intervals and the ED_{50} values are shown. Details of the DD test results can be found in Table 16 in Appendix B. LSD-like DS properties were observed for each analogue and all of the 8 rats tested with saline selected the saline lever. In terms of quantitative activity, the test drugs can be divided into three groups based on non-overlapping 95% confidence intervals. LSD was clearly the most potent while DOM was of intermediate potency, and the 4-butyl derivatives were least potent. At least one dose of each test drug produced DS effects that were indistinguishable from those of LSD, in at least 7/8 rats. With the exception of the two least potent compounds, 25 and *S*-25, a significant number of disruptions at the doses which mimicked the training dose of LSD was not observed.

Table 8. Potency of 4-alkyl-2,5-DMA derivatives in LSD-trained rats.[a]

Compound	ED$_{50}$(μM/kg)	95% C.I.	Potency Relative to DOM	%D[b]
LSD	0.02	0.01-0.04	3000	0
DOM	0.61	0.38-0.95	100	0
24	2.30	1.48-3.58	27	0
R-25	3.08	2.20-4.31	20	9
25	3.69	2.24-6.08	16	23
S-25	4.76	3.06-7.42	13	29

[a] The training dose of LSD tartrate was 0.08 mg/kg (0.186 μmol/kg). See Chapter 1 for complete experimental details.

[b] The percentage of the total number of rats tested at the dose which substituted for LSD that did not finish 50 presses in 5 minutes.

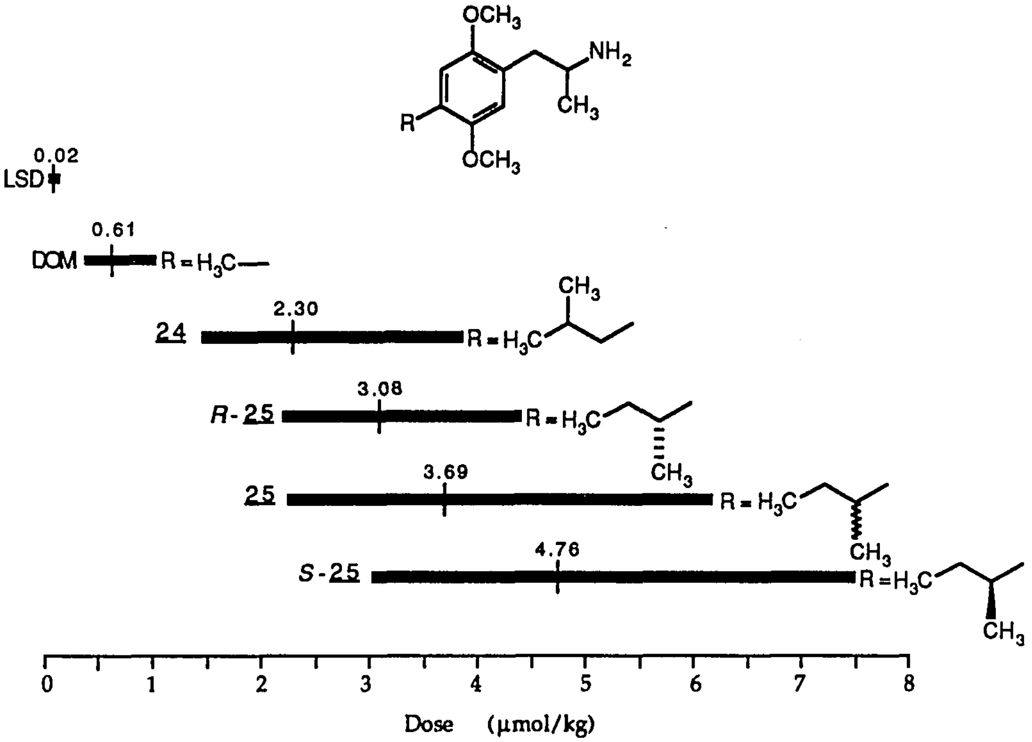


Figure 29. Potency diagram of 4-butyl-2,5-DMA derivatives.

In the DD paradigm, the practical limitations on the number of rats that are tested per dose of test compound results in large confidence intervals and a loss of statistical significance for relatively small differences (e.g. less than 2 fold) in potency. However, a trend of relative activity is apparent within this group of 4-butyl derivatives.

The ED_{50} values for 24 and 25 suggest that the isobutyl substituent is associated with slightly higher potency, relative to the secbutyl substituent. Analysis of the data by the method of Litchfield and Wilcoxon (1949) indicated that a significant potency difference only exists between 24 and *S*-25 ($PR > f_{PR}$), but not between the other 4-butyl derivatives. A modest degree of stereoselectivity was observed in the results for *R*- and *S*-25. The eudismic ratio for 25, that is the potency of the eutomer (the *R*-isomer) divided by the potency of the distomer (the *S*-isomer) is about 1.5.

Conclusions

The major conclusion of this study is that branching in the 4-alkyl group of DOM analogues leads to compounds which retain activity, but with decreased potency. The position and stereochemistry of a methyl branch, added to the *n*-propyl group of DOPR, probably has a relatively minor influence on the extent to which the high potency of this compound is attenuated. Before discussing these findings, an important methodological issue, involving the validity of using LSD-trained rats to evaluate the activity of phenethylamine-type hallucinogens, deserves some brief attention. The critical point is that inferences of steric effects on drug-receptor interactions, from behavioral data that are sensitive exclusively to LSD-like DS properties, are valid only if the same receptor(s) is involved in both actions.

It can be argued that LSD and DOM (along with mescaline) belong to different chemical classes of hallucinogens, possessing non-identical *in vitro* pharmacology, i.e. relative binding affinities to various subtypes of serotonin receptors (see references in Titeler, et al., 1988). LSD seems to function as a compound stimulus with elements that possibly reflect distinct receptors (Winter and Rabin, 1988). The DS properties of the test compounds that rats perceive as similar to LSD may be associated with binding at any one of several different sites.

However, the results from this and other studies strongly suggest that the evaluation of DOM analogues in LSD-trained rats is valid. For example, previous DD studies provide consistent evidence supporting a common activity for LSD and phenethylamine hallucinogens in rats trained on LSD (Appel and Cunningham, 1986), DOM (Glennon et al., 1986a) and mescaline (Winter, 1978). Recently, Winter and Rabin (1988) have argued that the salient element of the LSD cue is related to DOM-like actions (possibly mediated by 5-HT_2 receptors). A second element (possibly mediated by 5-HT_1 receptors) that is not shared with DOM, is apparent only under special conditions.

As discussed in Chapter 1, important questions remain to be answered before the identity of these critical binding sites is conclusively established and well understood. In terms of our present level of understanding, if a single receptor type mediates the major effects of hallucinogens, the 5-HT_2 receptor appears to be the most likely candidate (Pierce and Peroutka, 1989; Titeler et al., 1988). In that case, the inability of DOET and DOPR to substitute for 5-MeO-DMT (Glennon et al., 1981a), discussed in the introduction section, may relate to greater involvement of 5-HT_{1A} receptors in the behavioral properties of 5-MeO-DMT (Spencer et al., 1987). Thus, although the DS properties of LSD and DOM are very similar, more work is clearly needed to elucidate the comparative DS properties of 5-MeO-DMT.

The results of this study indicate that all of the 4-alkyl-2,5-DMA analogues are able, at different doses, to mimic the DS properties of LSD. Furthermore, the LSD to DOM potency ratio that was observed in the present set of stimulus generalization tests (30/1) is comparable to those obtained in previous DD studies in which LSD (Winter and Rabin, 1988), and DOM (Glennon et al., 1983) were employed as training drugs. This suggests that the DS properties (and the receptor interactions which mediate them) that are shared by LSD and DOM, are also apparent in the actions of 24, 25, *R*-25, and *S*-25.

The lack of behaviorally disruptive effects for the more potent test drugs provides further evidence that their psychopharmacology resembles that of LSD. DOM, 24 and *R*-25 completely substituted for the training dose of LSD at doses which produced no disruption of operant behavior. This would be expected if actions at a common receptor, or population of receptors, mediate their DS properties. Large numbers of disruptions generally indicate an incomplete overlap of DS properties, and may be a consequence of those drug actions that are not shared with the training drug (Stolerman and D'Mello, 1981). Therefore, LSD-stimulus-generalization to the 4-alkyl-2,5-DMA derivatives probably occurs through mechanisms (i.e. drug receptor interactions) that are also associated with the actions of DOM. These combined arguments establish the validity of using LSD-substitution tests in this study.

The results obtained from these DD tests clearly demonstrate the attenuation of activity resulting from branching in the 4-alkyl substituent and can be interpreted with respect to steric effects on the drug-receptor interaction. The complete substitution for LSD by all four butyl derivatives indicates that alkyl-branching of the 4-substituent of DOM analogues does not necessarily abolish activity. However, the mere presence of branching decreases potency by a factor of 4-8-fold, as calculated from the ED_{50} values.

This is consistent with the 6-fold decrease in potency, relative to DOM, that was observed in the rabbit hyperthermia model for the similarly branched 4-isopropyl derivative (Aldous et al., 1974).

The present results provide evidence that activity is somewhat sensitive to the position and stereochemical configuration of the branching. The 4-isobutyl derivative 24, appears to be about 60% more potent than the 4-secbutyl derivative 25. This may reflect the decreased ability of the 4-alkyl group of 24, relative to 25, to sterically hinder the approach of the electron-rich phenyl ring to the receptor surface. Branching adjacent to the ring, as in 25, especially when in the *S*-configuration, is associated with decreased potency and greater numbers of disruptions. Evidence for the stereochemical effects of branching is seen in the significantly higher potency of 24 relative to *S*-25, but not *R*-25.

Aldous et al. (1974) noted steric restrictions on the 4-substituent in the rabbit hyperthermia model since the 4-isopropyl derivative was more potent than the 4-tertbutyl analogue, DOTB. Additional studies with DOTB, which contains a more highly hindered benzylic carbon, indicate that hallucinogen-like activity may actually be abolished in man (Shulgin and Dyer, 1975) and drastically attenuated in animals (Glennon et al., 1982). This suggests that as steric hindrance to the drug receptor interaction increases, with the introduction of bulkier alkyl groups at the 4-position, potency decreases to the point where hallucinogenic activity is abolished. This would be expected if charge-transfer complex formation, or some other sterically-sensitive mechanism, is involved in the drug-receptor interaction.

The greater activity of *R*-25, relative to *S*-25, is consistent with the suggestion that it may be the α-molecular face (that which faces the benzene ring in Figure 23) which interacts with the receptor. This is based on the results of the analysis (Nichols and Weintraub, 1982) discussed earlier and agrees with a prediction made from comparisons with LSD, DOM, and several active and non-active DOM analogues (Weintraub and Nichols, 1978; Nichols and Glennon, 1984). However, the strength of such a conclusion is diminished by the modest degree of stereoselectivity that was observed.

The low eudismic ratio may indicate that the chiral carbon is some distance removed from the portion of the drug that is critical to the drug-receptor interaction (Ariëns, 1984). In that case, the relatively small differences in the observed potencies for 24, *R*,*S*-25, *R*-25, and *S*-25 may accurately reflect the ancillary role of whatever binding mechanism is involved. That is, if the critical event in the receptor stimulation by agonist molecules is mediated by mechanisms sensitive to these types of steric effects, a much greater potency difference would be expected. But, if the critical part of the interaction is going on elsewhere in the molecule, and the formation of a π-complex, for example, merely facilitates that event, then potency variations not unlike those observed in the present study would make sense. This possibility was recently discussed by Kolb (1987):

> In the case of hallucinogens, the charge transfer from the aromatic moiety makes an electrostatic interaction of this moiety with the receptor possible, but such an interaction may be just a component of the overall binding, a process in which the receptor recognizes and captures the drug. The nitrogen from the nonaromatic portion, such as the side chain (or its equivalent), which is believed to interact with the receptor in its protonated form, may carry more relative importance in eliciting the biological response than the aromatic moiety, although both are necessary for activity. One could speculate, for example, that the role of the aromatic moiety is to anchor the drug to the receptor and that the biological response is triggered by, e.g., proton transfer from the nitrogen from the side chain to the receptor. As the putative receptors for hallucinogens become better defined, some of these questions may be answered.

Therefore, if the various 4-alkyl substituents of 24, 25, *R*-25, and *S*-25 hinder, to various degrees, a relatively minor component of the drug-receptor interaction, greater differences in steric properties may be required before significant quantitative effects are observed in activity. The lack of activity for DOTB might reflect such a case. Alternatively, similar steric influences may have profoundly greater effects on activity if they were to occur at a more critical portion of the molecule. The carbon α to the nitrogen of hallucinogenic amphetamines, for example, is well known to exhibit sensitivity to both steric and stereochemical factors (Nichols, 1981).

In addition to influencing activity through indirect mechanisms involving the phenyl ring, direct interactions between the receptor and the 4-substituent have also been proposed (Nichols and Glennon, 1984). For example, it was suggested that the binding site may possess a specific hydrophobic region that accomodates the complementary 4-substituent when it extends out from the aromatic ring by no more than 5-6 Å (Nichols et al., 1977). Other workers have come to similar conclusions (Green et al., 1978; DiPaolo, et al., 1978).

If such a hydrophobic site exists, a relationship should be demonstrable between the lipophilicity of the 4-substituent (π_4), which is postulated to be related to receptor binding forces, and human hallucinogenic activity. Indeed, this was found to be the case for molecules with lipophilic 4-substituents, suggesting that chemical groups at this position of the aromatic ring might provide a distinct third site in the drug-receptor interaction (Domelsmith et al., 1981).

Thus, when considered together with the steric constraints of such a binding site, π_4 could account for much of the variation in the activity of the 4-alkyl-2,5-DMA analogues. However, it appears that the simple presence of any group enhances activity in a noncontinuous way (Nichols et al., 1984b; Nichols

1986a). For example, the potent analogue DON possesses a hydrophilic nitro group at the 4-position (Gomez-Jeria et al., 1987). If hydrophobic binding of the 4-substituent was critical, DON would not be expected to be of high potency unless other factors make the nitro group unusual.

The enhancement of receptor binding by the 4-substituent may be related to modulation of the electron distribution in the aromatic ring along with direct interactions with a complementary area of the receptor surface (Gomez-Jeria et al., 1987). Thus, the decreased lipophilicity of DON, resulting in the loss of optimal distribution properties, may be compensated for by electronic factors that substantially increase receptor affinity (Gomez-Jeria et al., 1987). Such factors could presumably compensate for the inability of the nitro substituent to participate in hydrophobic bonding as well.

Other speculative qualitative models visualize the receptor as a groove or cavity in the cell membrane, or with a region which normally accomodates the 4-substituent, that lies perpendicular to the aromatic ring (Nichols and Glennon, 1984). Both of these models are also consistent with the data observed here. In the first case, steric bulk both above and below the face of the molecule may inhibit binding to only slightly different degrees. In the second case, any branching in the 4-alkyl group may present similar steric interactions with a "curved" receptor (Nichols and Glennon, 1984).

An alternative hypothesis has recently been proposed which attributes the role of the 4-alkyl group to a potentially critical intramolecular interaction. The 4-substituent might present a steric boundary that forces the 5-methoxy to adopt a conformation that optimizes the directionality of the electron pairs of the methoxy oxygen (Nichols, et al., 1984b). This concept is also consistent with the data obtained in this study. That is, if the lone pair electrons of the 5-methoxy need to be directed toward the 4-substituent, branching in the alkyl group at that position could pose significant steric problems for their interaction with the receptor. This would conceivably interfere with binding, despite the fact that this substituent adequately influences the 5-methoxy to remain correctly directed away from the *para*-position.

In conclusion, the results of the present study provide further evidence of the deleterious effect on the activity of 2,5-DMA analogues due to branching in the 4-alkyl substituent. The position and stereochemistry of the branching exert some influence, but do not appear to be critical factors in this SAR. Additional studies will be necessary to elucidate the mechanism by which this type of branching attenuates activity. Further investigations could eventually lead to a better understanding of the specific features of serotonin receptors that influence ligand binding.

Experimental Section

1-(2,5-Dimethoxyphenyl)-2-methyl-1-propanone, **28** To a vigorously stirring solution of 4.56 g (30 mmol) of p-dimethoxybenzene 26 and 3.20 g (30 mmol) of isobutyryl chloride 27 in 50 mL of dry methylene chloride, cooled in an ice bath, was added 7.8 g (33 mmol) of $SnCl_4$ dropwise over 45 min. When the addition was complete the reaction mixture was allowed to warm to room temperature (30 min) and was then heated at reflux on a steam bath for 2 h. The mixture was then cooled and poured over crushed ice (10 g), and the methylene chloride layer was washed with 2 x 50 mL of 6 N HCl, followed by 2 x 100 mL of water, and was then dried (Na_2SO_4) overnight. The solution was filtered, and the filtrate was concentrated by rotary vacuum evaporation. The yel-low residue was purified by vacuum distillation to afford 4.48 g (72%) of the ketone: bp 88° C (0.04 mm); ^{1}H-NMR ($CDCl_3$) δ 1.14 (d, 6, CH_3, J = 6.9 Hz), 3.44 (m, 1, CH, J = 6.9 Hz), 3.77 (s, 3, OCH_3), 3.82 (s, 3, OCH_3), 6.93 (m, 3, ArH); Anal. ($C_{12}H_{16}O_3$) C,H.

2,5-Dimethoxy-α-(1-methylethyl)-benzenemethanol, 29. The reduction of 4.48 g (21.5 mmol) of 28 was accomplished by treatment with 0.81 g (21.5 mmol) of sodium borohydride in 25 mL of dry ethanol. The mixture was stirred at room temperature for 2.5 h and was then concentrated under vacuum. The residue was partitioned between methylene chloride and water and the organic phase was reduced by rotary vacuum evaporation. The resulting light yellow oil was purified by vacuum distillation to yield 3.25 g (72%) of the alcohol: bp 97°C (0.05 mm); ^1H NMR ($CDCl_3$) δ 0.80 (d, 3, CH_3, $J = 6.7$ Hz), 1.01 (d, 3, CH_3, $J = 6.7$ Hz), 2.00 (m, 1, CH), 2.48 (d, 1, OH), 3.77 (s, 3, OCH_3), 3.79 (s, 3, OCH_3), 4.48 [t, 1, C(OH)H], 6.80 (m, 3, Ar H); Anal. ($C_{12}H_{16}O_2$) C,H.

1,4-Dimethoxy-2-(2-methyl-1-propenyl)-benzene, 30. To a solution of 4.5 g (21.4 mmol) of 29 in 50 mL of benzene was added 0.4 g of Amberlyst-15 cation-exchange resin. After the solution was heated at reflux for 1.5 h, the resin was filtered off, and the benzene was reduced under vacuum, yielding 3.9 g (95%) of the crude alkene. The product was purified by vacuum distillation: bp 92°C (0.06 mm); ^1H-NMR ($CDCl_3$) δ 1.81 (s, 3, CH_3), 1.92 (s, 3, CH_3), 3.74 (s, 6, OCH_3), 6.28 (s, 1, =CH), 6.78 (m, 3, Ar H); Anal. ($C_{12}H_{16}O_2$) C,H.

1,4-Dimethoxy-2-(2-methylpropyl)-benzene, 31. A solution of 1 g (5.2 mmol) of the alkene 30 in 40 mL of ethanol containing 150 mg of 5% Pd/C was shaken under under an H_2 atmosphere at an initial pressure of 50 psig for 3 h. Concentration of the filtrate after removal of the catalyst gave a quantitative yield of 31 which was vaccum distilled: bp 72°C (0.4 mm); ^1H-NMR ($CDCl_3$) δ 0.90 (d, 6, CH_3, $J = 6.7$ Hz), 1.90 (m, 1, CH, $J = 6.7$ Hz), 2.45 (d, 2, CH_2, $J = 7.2$ Hz), 3.76 (s, 6, OCH_3), 6.76 (m, 3, Ar H); Anal. ($C_{12}H_{18}O_2$) C, H.

2,5-Dimethoxy-4-(2-methylpropyl)-benzaldehyde, 32. A solution of 0.5 g (2.58 mmol) of 31 and 1.34 g (5.16 mmol) of $SnCl_4$ in 20 mL of dry methylene chloride was stirred and cooled in an ice bath to 5°C. Dropwise addition of 0.45 g

(3.87 mmol) of Cl_2CHOCH_3 over 30 min was followed by reflux for 3 h. After cooling, the reaction mixture was poured over 5 g of ice and the methylene chloride layer was then washed with 3 x 30 mL of 6 N HCl and 3 x 30 mL of water. Drying (Na_2SO_4), filtering, concentrating, and purifying by vacuum distillation afforded 0.4 g (70%) of pure aldehyde: bp 104 °C (0.15 mm); ^{1}H-NMR ($CDCl_3$) δ 0.91 (d, 6, CH_3, J = 6.5 Hz), 1.90 (m, 1, CH), 2.53 (d, 2, CH_2, J = 7.0 Hz), 3.80 (s, 3, OCH_3), 3.88 (s, 3, OCH_3), 6.74 (s, 2, Ar H), 10.30 (s, 1, aldehyde H) Anal. ($C_{13}H_{18}O_3$)C, H.

1,4-Dimethoxy-2-(1-methylpropyl)-5-(2-nitro-1-propenyl)-benzene, 33. A mixture of 3.79 g (17 mmol) of the aldehyde 32 and 1.3 g (17 mmol) of ammonium acetate in 50 mL of nitroethane was heated on a steam bath for 2.5 h. The remaining nitroethane was removed by rotary vacuum evaporation, and the residue was partitioned between methylene chloride and water. The organic layer was concentrated, and the product was recrystallized from methanol to afford 3.12 g (66%) of yellow needles: mp 72° C; ^{1}H-NMR ($CDCl_3$) δ 0.91 (d, 6, CH_3, J = 6.5 Hz), 1.93 (m, 1, CH), 2.41 (d, 3, CH_3, J = 1 Hz), 2.51 (d, 2, CH_2, J = 7.1 Hz), 3.78 (s, 3, OCH_3), 3.83 (s, 3, OCH_3), 6.70 (s, 1, Ar H), 6.77 (s, 1, Ar H), 8.28 (s, 1, =CH); Anal. ($C_{15}H_{21}NO_4$) C, H, N.

2,5-Dimethoxy-α-methyl-4-(2-methylpropyl)-benzeneethanamine, 24. A solution of 1.24 g (32.4 mmol) of lithium aluminum hydride in 100 mL of dry ether was stirred while 3.0 g (10.8 mmol) of 33, dissolved in 25 mL of dry ether, was added dropwise over 10 min. The mixture was then heated at reflux on a steam bath for 2h. After cooling in an ice bath, the lithium aluminum hydride was quenched by addition of 3.72 mL of water in 10 mL of THF and allowed to stir for 3 h at room temperature. The precipitate was filtered and the ether was washed with 2 x 30 mL of water and dried (Na_2SO_4) overnight. The ether was removed by rotary vacuum evaporation and the free base was dissolved in 21 mL of 0.5 N ethanolic HCl. Recrystallization from 2-propanol/ethyl acetate afforded 1.63 g (60%) of white crystals: mp 164-166° C; ^{1}H-NMR (D_2O) δ 0.88 (d, 6, CH_3, J = 6.5 Hz), 1.38 (d, 3, CH_3, J = 6.5 Hz), 1.71 (br s, 2, CH_2), 1.77 (m, 1, CH), 2.44 (d, 2, CH_2, J = 6.9 Hz), 2.98 (m, 1, CH), 3.75 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 6.61 (s, 1, Ar H), 6.69 (s, 1, Ar H), 8.30 (br s, 3, NH_3); Anal. ($C_{15}H_{26}NClO_2$) C, H, N.

2,5-Dimethoxyacetophenone 41. A solution of 100g (0.73 mmol) of *p*-dimethoxybenzene 26, and 60.33 g (0.77 mmol) of acetyl chloride 40, in 400 mL CH_2Cl_2, was stirred in an ice bath while 140 g (0.87 mmol) of $SnCl_4$ was added dropwise over 1 h. After heating at reflux overnight, the solution was cooled to room temperature and poured over 10 g of ice in a separatory funnel. The aqueous lower layer containing tin salts was removed. The CH_2Cl_2 was washed with 3 x 500 mL of water, 3 x 400 mL 6N HCl, and 2 x 200 mL saturated NaCl. The solution was then dried (Na_2SO_4), filtered, and reduced by rotary vacuum evaporation. The product was purified by vacuum distillation, affording 115.11 g (88%); bp 94° C (0.35 mm), Literature bp 96° (0.25 mm) (Anderson and Lee, 1980); ^{1}H-NMR ($CDCl_3$) δ 2.61 (s, 3, aceto-CH_3), 3.79 (s, 3, OCH_3), 3.87 (s, 3, OCH_3), 6.94-7.01 (m, 2, ArH), 7.30 (s, 1, ArH); Anal. ($C_{10}H_{12}O_2$) C, H.

(*Z*)-and (*E*)-1,4-Dimethoxy-2-(1-methyl-1-propenyl)-benzene, 42. Following the procedure of Schlosser et al. (1986), a solution of 20.33 g (54.76 mmol) of ethyl triphenylphosphonium bromide in 100 mL of dry THF was stirred in a flame-dried 250 mL three neck roundbottom flask equipped with mechanical stirrer, condenser and addition funnel with cooling in an ice bath. After the addition of 23.54 mL (54.8 mmol) of a 2.36 M solution of BuLi in hexane over 10 min and 20 min of additional stiiring, 9.85 g (54.76 mmol) of 2,5-dimethoxyacetophenone 41, was added dropwise over 15 min. The ice bath was removed and the mixture was stirred at room temperature for 61 h. The THF was removed by rotary vacuum evaporation and the residue was dissolved in 50 mL of $CHCl_3$ and filtered through celite. After rotary vacuum evaporation of the $CHCl_3$, the product was purified by flash chromatography, eluting with 5% EtOAc in hexanes to yield 5.79 g (55%) of olefin. Analysis of the NMR integrations of the methyl protons at δ 1.47 (*Z*-isomer) and 1.75 (*E*-isomer) indicated a 7/3 ratio of *(Z) / (E)* isomers, which were separated by spinning band distillation: *(Z)*-42: bp 58° C (0.18 mm); ^{1}H-NMR ($CDCl_3$) δ 1.47 (d, 3, CH_3, J=6.7 Hz), 1.98 (d, 3, CH_3, J = 1.5 Hz), 3.77 (s, 6, OCH_3), 5.59 (q, 1, H, J = 6.7 Hz), 6.63 (d, 1, ArH, J = 2.6 Hz), 6.73-6.86 (m, 2, ArH); *m/e* 193 (MH^+); Anal. ($C_{12}H_{16}O_2$) C, H. *(E)*-42: bp 62° C (0.18 mm); ^{1}H-NMR ($CDCl_3$): δ 1.75 (d, 3, CH_3, J = 6.7Hz), 1.96 (s, 3, CH_3), 3.77 (s, 3, OCH_3), 5.56 (q, 1, H, J = 6.7 Hz), 6.73-6.86 (m, 3, ArH); *m/e* 193 (MH^+); Anal. ($C_{12}H_{16}O_2$) C, H.

(+)-Monoisopinocampheylborane. Following the procedure of Brown et al. (1982), a 500 mL flame-dried round bottom flask was charged with 24.34 mL (243.4 mmol) of 10 M borane-methyl sulfide and 160 mL of Et_2O. While the solution stirred at room temperature 76.28 g (560 mmol) of (-)-α-Pinene (Aldrich, freshly distilled from LAH; bp 155° C; $[\alpha]_D$= -41.11°) was added dropwise at a rate such that the reaction mixture refluxed gently. Following the addition, the mixture was heated at reflux for 0.5 h, then 18.36 mL (121.7 mmol) of tetramethylethylene-diamine (TMED, freshly distilled from CaH_2) was added and the mixture remained at reflux for 0.5 h. The $2IpcBH_2{\cdot}TMED$ complex was crystallized by allowing the flask to remain in the oil bath after the heat was turned off so that the solution cooled slowly to room temperature (if crystals do not form, a gentle stream of nitrogen on the surface of the solution can help to initiate the crystallization, Dr. B. Singaram, personal communication). After sitting overnight at room temperature the flask was cooled in a freezer for 7 h to ensure complete crytallization. A modification of the original procedure was used to separate the solid complex from the solution (Dr. B. Singaram, personal communication). The $2IpcBH_2{\cdot}TMED$ complex is stable to air and does not require special handling. Therefore the crystalline material was simply filtered, washed well with pentane, and dried under vacuum (5 h at 0.1 mm) yielding 41.98 g (83%) of (+)-$2IpcBH_2{\cdot}TMED$; $[\alpha]_D$ = +67.43° (c=9.7, EtOH); mp 139-140° C (Lit $[\alpha]_D$ = +69.03; mp 140.5-141.5° C; Brown et al., 1982a). Liberation of free (+)-monoisopinocampheylborane was accomplished by a modified procedure of Brown and Singaram (1984). A solution of 41.7 g (100 mmol) of (+)-$2IpcBH_2{\cdot}TMED$ in 134 mL of Et_2O was stirred while 24 mL (195 mmol) of 8.13 M $BF_3{\cdot}OEt_2$ was added over 20 min. Stirring was continued at room tempertaure for an additional 2 h. The moisture-sensitive (+)-monoisopinocampheylborane was removed from the $2BF_3{\cdot}TMED$ as follows (P.V. Ramachandran, personal communication). A round hole was bored out of the center of a 24/40 rubber stopper so as to allow the tight fit of a flame-dried gas dispersion tube (ROBU micro-filter candle, Aldrich, 20 mm length, 9 mm diameter, 100-160 pore size) which was closed off with a rubber septum. An 18 gauge double-ended needle was inserted through the septum so that the tip of the needle came within 1 cm of the filter end inside the candle. The other end of the needle was inserted through a rubber septum, into dry 500 mL roundbottom flask which was vented to a bubbler. The 24/40 rubber stopper with the candle and needle was quickly sealed on to the flask containing the reaction mixture and nitrogen was used to force the (+)-IpcBH solution through the filter and into the clean flask. To facilitate this procedure, the flask was hand-held while the candle was slowly stirred in the slurry. When the filtration was complete, an additional 72 mL of Et_2O was added by syringe to the original flask and filtered as before. This procedure was repeated once more with another washing of 72 mL Et_2O. The combined filtrate was analyzed for (+)-$IpcBH_2$ by hydrolysis with 1:1:1

glycerol, water, and THF (see Brown et al., 1975) as the hydrolyzing mixture and found to be 0.75 M, (230 mL; 173 mmol, 86%); ^{11}B-NMR (decoupled) +22.4 (singlet); $[\alpha]_D$ = +38.72 (c=11.2, Et_2O) (Literature data for (-)-isomer: $[\alpha]_D$ = -39.93, c = 11.9, Et_2O, Brown and Singaram, 1984).

(2*R*,3*R*)-(-)-3-(2,5-Dimethoxyphenyl)-2-butanol, 45. Asymmetric hydroboration and oxidation were accomplished by the method of Brown and Singaram (1984). A solution of 5.5 g (28.7 mmol) of *(Z)*-42 was added to 40 mL (30 mmol) of (+)-$IpcBH_2$ in Et_2O cooled to -35° C in a constant temperature bath. The mixture was swirled after the addition and then allowed to stand with cooling for 60 h. The monoisopinocampheylborane intermediate had crystallized from the ethereal solution which was removed using a gas dispersion tube and two-ended needle as described for (+)-$IpcBH_2$. The solid borane was washed and filtered twice with 20 mL of Et_2O. The solid was cooled in an ice bath and 3 mL of EtOH was added dropwise over 10 min, followed by 10 min of additional stirring. Then 5.27 mL of 6N NaOH was added over 3 min, and stirring was continued for an additional 5 min. After the dropwise addition of 8.6 mL of 30% H_2O_2 over 5 min, the mixture was heated to 50° C, stirred for 1 h, then cooled in an ice bath. To this, 30 mL of Et_2O was added, and the aqueous layer was saturated with K_2CO_3 and separated. The ether layer was washed with 50 mL of H_2O and 30 mL of saturated NaCl. The combined aqueous washes were extracted with 2 x 50 mL Et_2O, which was combined with the original Et_2O and dried (Na_2SO_4). After filtration and rotary vacuum evaporation of the solvent, the product was purified by silica gel flash chromatography, eluting with 5% EtOAC in hexane, to afford 7.8 g (71.4%) of 45: $[\alpha]_D$ = -4.10° (c = 4.97, EtOH); ^{1}H-NMR ($CDCl_3$): δ 1.09 (d, 3, CH_3, J = 6.4 Hz), 1.26 (d, 3, CH_3, J = 7.2 Hz), 2.01 (br s, 1, OH, vanishes with D_2O), 3.19-3.32 (m, 1, H), 3.77 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 3.84-3.98 (m, 1, H), 6.67-6.82 (m, 3, ArH), *m/e* 211 (MH^+), 193 (MH^+ -H_2O), *m/e* 211

(MH^+), 193 (MH^+ - H_2O); Anal. ($C_{12}H_{18}O_3$) C,H. Enantiomeric excess = 82.5% as determined by gas chromatography analysis of the corresponding ester derived from the reaction with *(R)*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA, Brown et al., 1987). This was carried out on a Hewlett-Packard 5750 chromatograph using a 50 m methylsilicone packed column; isothermal T = 205° C Similar analysis of the alcohol which remained in the ethereal filtrate separated from the crystallized monoisopinocampheylalkylborane indicated a 62% ee.

(-)-Monoisopinocampheylborane. Following a procedure identical to that used for the (+)-isomer, 61.3 g (450 mmol) of (+)-α-pinene (Aldrich, freshly distilled from LAH; bp 155°; $[\alpha]_D$ = +44.56°) was reacted with 19.6 mL (196 mmol) of borane-methyl sulfide and 14.75 mL (98 mmol) of tetramethylethylenediamine (TMED, freshly distilled from CaH_2) to afford 19.17 g (47%) of (-)-2IpcBH_2·TMED complex; [α] = -67.65° (c=9.6, EtOH); mp 139-140° C (Literature values for the (+)-isomer: $[\alpha]_D$ = +69.03; mp 140.5-141.5° C; Brown et al., 1982a).

Liberation of free (-)-monoisopinocampheylborane was accomplished with 18.9 g (45 mmol) of (-)-2IpcBH_2·TMED in 61 mL of Et_2O, and 10.9 mL (89 mmol) of BF_3·OEt_2. The solid 2BF_3·TMED complex was washed with an additional 2 x 33 mL of Et_2O, as described for the (+)-isomer. The resulting solution was analyzed for (-)-IpcBH_2 by hydrolysis with 1:1:1 glycerol, water, and THF (see Brown et al., 1975) as the hydrolyzing mixture and found to be 1 M, 68 mL (68 mmol, 76%); ^{11}B NMR (decoupled) +22.4 (singlet); $[\alpha]_D$ = -38.64 (c = 11.1, Et_2O) (Lit, $[\alpha]_D$ = -39.93, c=11.9, Et_2O, Brown and Singaram, 1984).

(2*S*,3*S*)-(+)-3-(2,5-Dimethoxyphenyl)-2-butanol, 46. Starting with 9 g (46.9 mmol) of *(Z)*-42 a procedure similar to that described for the preparation of 45 was used except that (-)-IpcBH_2 replaced the (+)-isomer. The purified yield of 46 was 6.9 g (70%); $[\alpha]_D$ = +4.18° (c = 5.26, EtOH); Enantiomeric excess = 82.9%

by GC analysis of MTPA ester; ^{1}H-NMR ($CDCl_3$) was identical to 45; *m/e* 211 (MH^+), 193 (MH^+ - H_2O); Anal. ($C_{12}H_{18}O_3$) C, H.

(2*R*,3*R*)-(-)-3-(2,5-Dimethoxyphenyl)-2-butyl-*p*-toluenesulfonate.

Tosylation was accomplished by the method of Kabalka et al. (1986). A solution of 7.4 g (35.24 mmol) of 45 in 35 mL of $CHCl_3$ was cooled in an ice bath and stirred while 5.7 mL (70 mmol) of pyridine was added, followed by 10 g (52.86 mmol) of *p*-toluenesulfonyl chloride in small portions over 10 min. After 3 h, 100 mL of Et_2O and 25 mL of H_2O were added and the organic layer was washed with 100 mL of 2 N HCl, 100 mL 5% $NaHCO_3$, 100 mL of H_2O and dried over $MgSO_4$. The solution was filtered and the solvent was removed by rotary vacuum evaporation. The tosylate was purified by silica gel flash chromatography eluting with 5% EtOAc in hexane to afford 8.79 g (69%); $[\alpha]_D$ = -25.14° (c = 5.39, EtOH); ^{1}H-NMR ($CDCl_3$) δ 1.17 (d, 3, CH_3, J = 7.0 Hz), 1.25 (d, 3, CH_3, J = 6.4 Hz), 2.41 (s, 3, ArCH3), 3.21 (pentet, 1, J = 7.0 Hz), 3.69 (s, 3, OCH3), 3.72 (s, 3, OCH_3), 4.82 (pentet, 1, CH, J = 6.4 Hz), 6.59 (s,1,ArH), 6.65 (s, 2, ArH), 7.21 (d, 2, ArH); *m/e* 193 (MH^+ - TsOH); Anal. ($C_{19}H_{24}SO_5$) C, H;

S-(+)-1,4-Dimethoxy-2-(1-methylpropyl)-benzene, S-(+)-36. To a solution of 7.8 g (21.43 mmol) of the (2*R*,3*R*)-tosylate, dissolved in 10 mL of dry ether, while stirring under nitrogen with cooling in an ice bath, was added over 2 min, 40 mL of a 1 M solution of lithium aluminum hydride in THF. After an additional 5 min of stirring, the ice bath was removed and the mixture was heated at reflux for 5 h. An additional 50 mL of ether was added and the mixture was again cooled in an ice bath. With vigorous stirring, the lithium aluminum hydride was quenched by addition of 3.2 mL of water, and stirring was continued at room temperature for 20 min. The precipitate was filtered through celite and the ether was washed with 2 x 50 mL of 3 N HCl, 2 x 50 mL of 5% $NaHCO_3$, and

50 mL of saturated NaCl. After drying ($MgSO_4$), filtration, and concentration by rotary vacuum evaporation, the crude product was purified by centrifugal chromatography using a 4 mm silica plate and eluting with 1% EtOAc in hexane, to afford 2.5 g (60%) of *S*-(+)-36: $[\alpha]_D$ = +16.63° (c=5.01, EtOH); ^{1}H-NMR ($CDCl_3$) δ 0.84 (t, 3, CH_3, J = 7.3 Hz), 1.18 (d, 3, CH3, J = 7.0 Hz), 1.48-1.62 (m, 2, CH_2), 3.07 (m, 1, H, J = 7.0 Hz), 3.77 (s, 6, OCH_3), 6.63-6.80 (m, 3, ArH); *m/e* 195 (MH^+); Anal. ($C_{12}H_{18}O_2$) C, H.

(2*S*,3*S*)-(+)-3-(2,5-Dimethoxyphenyl)-2-butyl-*p*-toluenesulfonate. A procedure similar to that described for the (2*R*,3*R*)-isomer was used, starting with 6.7 g (31.9 mmol) of (2*S*,3*S*)-alcohol 46, which afforded 8.25 g (71%) of the tosylate: $[\alpha]_D$ = +27.13° (c = 5.5, EtOH); the ^{1}H-NMR spectra of the two tosylate enantiomers were identical: *m/e* 193 (MH^+ - TsOH); Anal. ($C_{19}H_{24}SO_5$) C, H.

***R*-(-)-1,4-Dimethoxy-2-(1-methylpropyl)-benzene, *R*-(-)-36.** Using an identical procedure to the one used for the *S*-isomer, the reduction of 8.00 g (21.97 mmol) of the (2*S*,3*S*)-tosylate afforded 2.17 g (51%) of *R*-(-)-36: $[\alpha]_D$ = -17.46° (c=5.23, EtOH); The ^{1}H-NMR ($CDCl_3$) spectrum was identical to that of *S*-(+)-36; *m/e* 195 (MH^+); Anal. ($C_{12}H_{18}O_2$) C, H.

***R*-(-)-36-Alternate Method.** Following the procedure of Johnson and Dutra (1973) a solution of 13.8 g (100 mmol) of *p*-dimethoxybenzene 26, in 100 mL of dry THF was stirred at -78° C while 0.12 mmol of nBuLi in 48 mL of hexane was added over 10 min. The solution was then heated at reflux for 2 h, after which the solvent was removed by distillation, the flask was cooled in an ice bath, and 35 mL of dry Et_2O was added. A small aliquot was removed and analyzed in D_2O by ^{1}H-NMR, which confirmed that lithiation had occured. The diaryllithium solution was added dropwise over 1 h to a suspension of freshly purified CuI (Kauffman et al., 1983) in 30 mL of Et_2O in an ice bath. The mixture was then cooled further in an acetone/dry ice bath and 4 g (17 mmol) of *S*-(+)-2-butyl tosylate (Johnson and Dutra, 1973) in 25 mL of Et_2O was added dropwise over 30 min, followed by overnight stirring. The specific rotation of the tosylate used indicated that the optical purity was 69% since the $[\alpha]_D$ was found to be identical (+9.9°, c=5, EtOH), to the $[\alpha]_D$ reported for the same compound of 69% ee in the literature (Johnson and Dutra, 1973). The reaction was then quenched with 50 mL of a saturated solution of NH_4Cl added over 10 min and was stirred for an additional 15 min. The Et_2O layer was separated, washed with 3 x 100 mL of a saturated NaCl solution, and dried over $MgSO_4$. The solution was filtered and the solvent was removed under vacuum. Purification of the product was accomplished in two steps, beginning with flash chromatography over silica and elution with 40% Et_2O in hexanes. Finally, centrifugal chromatography (Chromatotron) on a 4 mm silica rotor, eluting with 20% Et_2O in hexanes, afforded 1.25 g (38%) of *R*-(-)-36; $[\alpha]_D$ = -12.94° (c=5, EtOH). The negative sign of rotation for the *R*-isomer confirms the assignment of absolute configuration inferred from the mechanism of the hydroboration reaction described above. The decreased rotation value for this product resulted from the low optical purity of the *S*-(+)-2-butanol obtained from Aldrich. The ^{1}H-NMR spectrum of this product was identical to that of the same product prepared through the asymmetric hydroboration/oxidation method.

***S*-(+)-2,5-Dimethoxy-4-(1-methylpropyl)-benzaldehyde, *S*-(+)-47.** A solution of 770 mg (3.97 mmol) of *S*-(+)-36 in 20 mL of dry methylene chloride was stirred under nitrogen in an ice bath for 10 min. After addition of 1.55 g (5.96 mmol) of $SnCl_4$, 685 mg (5.96 mmol) of Cl_2CHOCH_3 was added. The ice bath was removed and following 10 min of additional stirring, 5 g of ice was added. The mixture was directly loaded on to a small silica flash column and eluted with methylene chloride. The crude product was purified by centrifugal chromatography on a 4 mm silica plate and elution with 5% EtOAc in hexane to afford 764 mg (87%) of

the aldehyde: $[\alpha]_D$ = +18.6 (c=5.53, EtOH); ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3, CH_3, J= 7.3 Hz), 1.21 (d, 3, CH_3, J = 7.0 Hz), 1.52-1.68 (m, 2, CH_2), 3.15 (m, 1, H, J = 7.0 Hz), 3.82 (s, 3, OCH_3), 3.90 (s, 3, CH_3), 6.81 (s, 1, ArH), 7.28 (s, 1, ArH), 10.40 (s, 1, CHO); *m/e* 223 (MH^+); Anal. ($C_{13}H_{18}O_3$) C, H.

Alternate route. Following a modified procedure of Weyerstahl et al. (1970) a solution of 0.6 g (3.1 mmol) of *S*-(+)-36, identical to the material used above, was dissolved in 15 mL of acetic acid and was stirred at room temperature while 0.55 g (3.4 mmol) of iodine monochloride was added over 2 min. After stirring for 2 h, the reaction mixture was diluted with 30 mL Et_2O and washed with 3 x 30 mL of 5% $NaHCO_3$, 3 x 30 mL 5% $Na_2S_2O_3$, and 3 x 30 mL of saturated NaCl. The solution was dried (Na_2SO_4), filtered, reduced by rotary vacuum evaporation, and purified by chromatotron (2mm silica rotor eluting with CH_2Cl_2) to afford 679 mg (68%) of 4-iodo-*S*-(+)-36: $[\alpha]_D$ = +15.59° (c=1, EtOH); ^{1}H-NMR ($CDCl_3$) δ 0.84 (3,t, CH_3), 1.17 (3, d, CH_3), 1.55 (2, m, CH_2), 3.02 (1, q, CH), 3.76 (3, s, OCH_3), 3.83 (3, s, OCH_3), 6.67 (1, s, ArH), 7.20 (1, s, ArH).

A solution of 675 mg (2.11 mmol) of this iodinated material in 5 mL of dry THF was stirred under N_2 and cooled to -78° C. A solution of 2.32 mmol (0.98 mL of 2.36 M solution) of *n*BuLi in hexane was then added over 3 min. The mixture was allowed to stir for an additional 3 min, after which 366 mg (5 mmol) of DMF was added. The reaction was complete in 30 min, as determined by tlc, at which time 10 mL of saturated NH_4Cl was carefully added aver 20 min, followed by 10 min of stirring. The mixture was then diluted with 30 mL of pentane and washed with 3 x 30 mL of saturated NaCl, dried (Na_2SO_4), filtered, and reduced by rotary vacuum evaporation. Purification was accomplished by chromatotron (2 mm silica rotor eluting with CH_2Cl_2) which afforded 120 mg (26%) of the *S*-(+)-aldehyde; $[\alpha]_D$ = +18.14° (c=4.75, EtOH), which is comparable to the value obtained for the same product obtained by the other formylation procedures; $[\alpha]_D$ = +18.6 (c=5.53, EtOH) for the product of the Cl_2CHOCH_3 reaction, described above, and $[\alpha]_D$ = +18.03 (c=4.52, EtOH) for the product of the Vilsmeier-Haack reaction. All three products were identical by tlc, R_f = 0.4 (silica, CH_2Cl_2) and ^{1}H-NMR.

R-**(-)-2,5-Dimethoxy-4-(1-methylpropyl)-benzaldehyde,** *R*-**47**. Using the first of the two procedures described for the *S*-isomer, 1.1 g (5.67 mmol) of *R*-36 was formylated and purified to yield 996 mg (79%) of the aldehyde: $[\alpha]_D$ = -18.54° (c=5.5, EtOH); The ^{1}H-NMR ($CDCl_3$) spectrum was identical to that of *S*-47; *m/e* 223 (MH^+); Anal. $C_{13}H_{18}O_3$) C, H.

S-**(+)-1,4-Dimethoxy-2-(1-methylpropyl)-5-(2-nitropropenyl)-benzene,** *S*-**(+)-48.** A mixture of 764 mg (3.44 mmol) of the (+)-aldehyde and 2.3 g (30 mmol) of ammonium acetate in 8 mL of nitroethane was heated at reflux under nitrogen for 4 h. The excess nitroethane was removed by rotary vacuum evaporation and the residue was partitioned between methylene chloride and water. After purification over a small silica gel flash column, and elution with methylene chloride, the product was further purified by centrifugal chromatography on a 4 mm plate, eluting with methylene chloride, to afford 879 mg (92%) of pure product: $[\alpha]_D$ = +17.07 (c=5, EtOH); ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3, CH_3, J=7.3 Hz), 1.21 (d, 3, CH_3, J=7.0 Hz), 1.52-1.65 (m, 2, CH_2), 2.43 (s, 3, CH_3), 3.13 (m, 1, H, J = 7.0 Hz), 3.80 (s, 3, OCH_3), 3.85 (s, 3, CH_3), 6.77 (s, 1, Ar H), 6.79 (s, 1, Ar H), 8.29 (s, 1, =CH); *m/e* 280 (MH^+), 223 (MH^+ - C_4H_9); Anal.($C_{15}H_{21}NO_4$) C, H, N.

R-**(-)-1,4-Dimethoxy-2-(1-methylpropyl)-5-(2-nitropropenyl)-benzene,** *R*-**(-)-48.** The con- densation of 996 mg (4.49 mmol) of the (-)-aldehyde with nitroethane was accomplished by the method described for the *S*-nitropropene to afford 819 mg (66%) of pure product: $[\alpha]_D$ = -17.71° (c=5, EtOH); The ^{1}H-NMR ($CDCl_3$) spectrum was identical to that of *S*-48; *m/e* 280 (MH^+), 223 (MH^+ -C_4H_9); Anal

($C_{15}H_{21}NO_4$) C, H, N.

***S*-(+)-2,5-Dimethoxy-α-methyl-4-(1-methylpropyl)-benzeneethanamine, *S*-(+)-23.** To a stir-ring, ice bath-cooled solution of 760 mg (2.72 mmol) of the (+)-nitropropene in 10 mL of dry ether under nitrogen, 10 mL of a 1 M solution of lithium aluminum hydride in THF was added over 5 min. The ice bath was removed and the mixture was stirred at reflux for 2.5 h. Additional ether was then added (30 mL) and the mixture was cooled in an ice bath and then quenched by addition of 0.5 mL of water. After stirring at room temperature for 15 min, the mixture was filtered through celite and the crude amine was extracted into 3 x 30 mL of 3 N HCl. Following basification with 5 N NaOH, the free base was extracted into 3 x 50 mL of ether and dried (Na_2SO_4). The drying agent was removed by filtration and the solvent was removed by rotary vacuum evaporation. The residue was acidified with HCl and recrystallized from acetonitrile to afford 394 mg (58%) of the hydrochloride salt: mp 162-164° C; $[\alpha]_D$ = +15.27° (c = 1.1, H_2O); ^{1}H-NMR ($CDCl_3$) δ 0.84 (t, 3, CH_3, J = 7.3 Hz), 1.16 (d, 3, CH_3, J = 6.7 Hz), 1.37 (d, 3, CH_3, J = 6.4 Hz), 1.51-1.61 (m, 2, CH_2, J = 7.0 Hz), 2.87-3.10 (m, 4, CH_2, CH, CH), 3.77 (s, 3, OCH_3), 3.80 (s, 3, OCH_3), 6.68 (s, 1, ArH), 6.70 (s, 1, Ar H), 8.37 (br s, 3, NH_3); *m/e* 252 (MH^+ - HCl); Anal. ($C_{15}H_{26}ClNO_2$) C, H, N.

***R*-(-)-2,5-Dimethoxy-α-methyl-4-(1-methylpropyl)-benzeneethanamine, *R*-(-)-25.** The reduction of 817 mg (2.93 mmol) of the *R*-(-)-nitropropene was carried out by the procedure described for *S*-(+)-25. Recrystallization from acetonitrile afforded 610 mg (83%) of the hydrochloride salt: mp 164 ° C; $[\alpha]_D$ = -15.74° (c=1, H_2O); The ^{1}H-NMR ($CDCl_3$) spectrum was identical to that of *S*-(+)-25; *m/e* 252 (MH^+ - HCl); Anal. ($C_{15}H_{26}ClNO_2$) C, H, N.

CHAPTER 4 - DRUG DISCRIMINATION STUDIES OF ENTACTOGENS

Introduction

The significance and goals of this work were presented in Chapter 1. The discussion that follows will be directed toward the rationale for proposing that entactogens be viewed as a distinct drug class and the experimental findings with which the validity of such a view has been examined. This topic will be approached through a traditional medicinal chemistry perspective in which one attempts to dissect relevant activities, and their pharmacological mechanisms, to the extent to which they are presently understood, in terms of SARs. Following this, progress toward the development of an "ideal" entactogen will be presented.

The compounds which comprise this recently proposed class of drugs are currently small in number and are closely related in chemical structure. They are ß-phenethylamine (PEA) derivatives with a 3,4-methylenedioxy aromatic substituent, either a single methyl group or unsubstituted on the nitrogen, and a methyl or ethyl substituent on the α-carbon (see Chapter 1 for structures).

As discussed in the first chapter, the *N*-methyl, α-ethyl derivative, MBDB, has been designated as the prototype entactogen because its effects are pharmacologically less complex (Nichols et al., 1986; Nichols, 1986). The "amphetamine" derivatives MDA and MDMA seem to exert a primary pharmacological activity similar to that of MBDB (Nichols, 1986). Along with MDA, MDMA and MBDB, the *N*-ethyl (MDE) and *N*-hydroxy (*N*-OH-MDA) derivatives may also possess entactogenic activity (Braun et al., 1980, Boja and Schechter, 1987).

Although PEA derivatives are chemically simple, a wide variety of physiological effects which they elicit have been studied. The parent compound, illustrated in Figure 30, occurs naturally in both plants and animals. It has been found in trace amounts in mammalian brain (Borison et al., 1976) and may function as a neuromodulator or neurotransmitter (Niddam et al., 1985). Because it is rapidly metabolized by monoamine oxidase Type B (MAO-B), its biological activity when administered exogenously is not significant unless that enzyme is inhibited (see references in Colpaert et al., 1980).

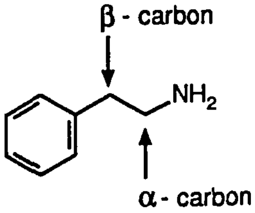


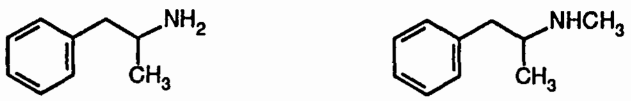
Figure 30. Molecular structure of ß-phenethylamine (PEA).

The addition of a methyl group to the α-carbon, as in "amphetamine" derivatives, seems to greatly attenuate the metabolism by MAO-B, leading to potent biological activity. Phenethylamine has behavioral activity in some animal models similar to amphetamine and has been conceptualized as an "endogenous amphetamine," but the evidence to support this hypothesis is equivocal (Goudie, 1982). The behavioral effects of cocaine have also been studied in terms of mediation through endogenous phenethylamine in the brain (Colpaert et al., 1980).

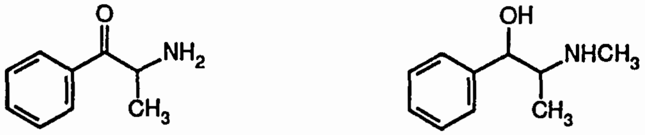
Some derivatives of PEA serve important biological functions, i.e. the catecholamine neurotransmitters, while a vast array of others have been synthesized and studied pharmacologically. The variety of effects produced by these molecules results from their interactions with many different biological sites. Some of these are in the brain and mediate behavioral effects, while others are found in peripheral organ systems such as the heart and kidney. In as much as the effects caused by bioactive molecules are mediated through different mechanisms and target sites, certain aspects of the SARs for the molecules producing these various activities contrast.

Consistent SARs emerge when individual compounds in a particular series are compared for a specific activity. The potency of each member in the series depends, to a large extent, on factors associated with the site of interaction. Each site may be envisioned as having distinct characteristics. Its location and environment will influence the type of chemical characteristics a potential ligand should have in order to obtain optimal access to that site. In addition, the chemical features of the molecules which compose the interactive surface of the target site will determine the optimum complementary chemical nature of the drug. Molecules which most closely conform to this optimum will tend to bind to the site with greater affinity. Since these factors are constant for a given binding site, different activities may be compared in terms of SARs. The existence of novel activity can be deduced if the observed SARs for the molecules producing it are sufficiently different from those associated with other activities. Employing this approach, experiments designed to test the hypothesis that entactogens possess novel activity were performed. The discussion that follows will center on a comparison of the molecular features of entactogens with those of the two most closely related classes of compounds, stimulants and hallucinogens.

The PEA derivative amphetamine, and its *N*-methyl homologue, methamphetamine (Figure 21), are the prototypical CNS stimulants. Amphetamine was first synthesized by Edeleano in 1887 and was independently resynthesized by Alles in 1927 in the search for antiasthmatic agents (Angrist and Sudilovsky, 1978). Its stimulant activity was not generally recognized until the 1930s when it began to be used in nasal inhalers in Germany. Naturally occurring stimulants such as ephedrine (found in *Ephedra vulgaris*, or ma huang in China), cathinone (found in *Catha edulis* or khat in Africa) and cocaine (found in *Erythroxylon coca* or coca in South America), have been used for centuries (Angrist and Sudilovsky, 1978). Cocaine is not a PEA, but it seems to produce stimulant effects through similar mechanisms.



AMPHETAMINE METHAMPHETAMINE



CATHINONE EPHEDRINE

Figure 21. Molecular structures of phenethylamine stimulants.

The stimulant effect in humans can generally be described as "wakefulness, alertness and a decreased sense of fatigue; elevation of mood, with increased initiative, self confidence, and ability to concentrate; often elation and euphoria; increase in motor and speech activity" (Weiner, 1980). Extensive research into the mechanism of action of amphetamine has led to the general conclusion that most of the stimulant effects are produced indirectly through the facilitation of dopamine release (Moore, 1978) from the neuronal cytoplasmic pool (Parker and Cubeddu, 1988).

Amphetamine is one of the oldest and most popular training drugs in DD experiments (Young and Glennon, 1986). The activity of amphetamine being measured in this assay, its discriminative stimulus or "cue" properties, seems to be related to the stimulant effect in humans. Results from several studies, for example, indicate that the cue is mediated primarily through dopaminergic systems (Ho and Silverman, 1978; Woolverton and Cervo, 1986; Young and Glennon, 1986). The low dose cue (0.6-1 mg/kg) of amphetamine may be mediated through mesolimbic DA systems, and the high dose cue (1.6 mg/kg), through striatal DA systems. (Neilsen and Jepsen, 1985).

By contrast, PEA hallucinogens produce a very different type of behavioral alteration which has been discussed in detail previously. Hallucinogenic PEA derivatives, such as the prototype, DOM (see chapter 3), contain aromatic methoxy substituents. The 3,4,5-trimethoxy derivative (mescaline, see chapter 1), although of relatively low potency, occurs naturally in the peyote cactus, used as a religious sacrament and medicine by native American Indians for centuries (McLaughlin, 1973; Stewart, 1987).

As discussed in Chapter 1, the cue produced by hallucinogenic training drugs seems to be related to 5-HT_2 agonist actions (see chapter 1). Stimulants and hallucinogens, therefore, produce distinct biological activities, mediated through different neurochemical mechanisms involving different sites of action and different SARs.

Several workers have employed DD to investigate the pharmacology of PEA (Colpaert et al., 1980; Goudie, 1982), as well as its stimulant (see Young and Glennon, 1986 for a review) and hallucinogenic (reviewed by Glennon et al., 1982a) derivatives. Until recently, entactogens have been studied as test drugs, in rats trained to discriminate either stimulants or hallucinogens from saline, but not as training drugs.

Substitution tests are extremely useful in DD experiments because a wide variety of compounds can be compared by testing for activity similar to the training drug, at the dose employed. The high specificity which is responsible for the power of this experimental design also sets limitations, since the results can only be used to compare drugs whose discriminable effects are essentially identical to the training drug (Overton, 1984). This feature was useful in the work described in the preceding chapters since we were concerned only with LSD-like effects. Potency was assessed by calculating ED_{50} values and this served as the basis for the various SAR studies described. If a particular drug did not completely substitute for LSD, it was fairly certain that whatever activity it had was not LSD-like, and it was unnecessary to do a more detailed analysis of its characteristics.

The study of entactogens, however, presented a different sort of challenge. Initially, substitution tests were performed with the racemic mixtures and individual optical isomers of MDA, MDMA and MBDB in rats trained to discriminate LSD from saline (Nichols et al., 1986b). As described later, the results provided evidence that the DS properties of entactogens differ significantly from those of LSD. But it was no longer sufficient to demonstrate a lack of similarity, since the non-LSD-like activity being studied was now the major focus. After formulating the hypothesis that entactogens were, in fact, unlike _any_ other class of pharmacological agents, it became apparent that the DD studies would have to be expanded to include additional training drugs.

The following discussion, therefore, begins with a section which describes the results of experiments with two groups of rats trained to discriminate either (±)-MDMA or (+)-MBDB from saline. The evidence for the similarity of the DS properties of entactogens will be presented and the mechanism of action will be discussed. Results from experiments with entactogen-trained rats, as well as with two additional groups of rats trained with LSD or (+)-amphetamine, are presented in the next two sections which address the differences between entactogens, hallucinogens, and stimulants. Finally, progress toward the development of an entactogen which could be clinically studied will be presented and directions for future studies will be outlined.

The Common Activity of Entactogens

The criteria by which a novel drug class can be established with Drug Discrimination experiments would seem to involve three major aspects. First, the compounds which exert the characteristic activity of this new class should produce similar DS properties. Evidence for this can be obtained in substitution experiments in which one member of the new class is demonstrated to cross substitute with other members. Also, since entactogens exist as chiral molecules, stereoselectivity should be consistent within the series.

Complete substitution should occur without producing a significant degree of behavioral disruption. The disruptive effects on bar-pressing, in substitution experiments, are generally recognized as an indication of actions other than those associated with the training cue. Stolerman and D'Mello (1981) have discussed "the commonly expressed view that drug-produced stimulus control is mediated by multiple effects of the agents concerned; drugs with only partially overlapping profiles of action might have to be given in relatively large doses to provide the shared effects with sufficient intensity."

Thus, in substitution tests with agents which share primary pharmacological effects, the dose of the test drug at which greater than 80% of the rats select the training drug lever might be expected to disrupt less than 20% of the rats tested. Complete substitution may occur with test drugs that share a secondary component of activity but such cases may be indentified when large numbers of disruptions are also observed. This concept is addressed later in this chapter.

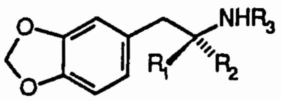
The second criteria involves the neurochemical mediation of the DS properties. Common transmitter systems should be involved for the cues produced by the individual members of the new class. Where secondary effects caused by involvement of other systems occur, evidence for this should be apparent from *in vitro* studies. The establishment of this criteria is somewhat more complex than the first since a number of different experiments are involved. These include substitution tests using standard agents with established mechanisms, pretreatments with standard antagonists, and certain other neuropharmacological manipulations.

The first two criteria will be addressed in this section. While the evidence for the similarity of the cues produced by MDA, MDMA, and MBDB is reasonably convincing at this time, the set of experiments needed to statisfy the second criteria is still in progress. The results accumulated thus far support a common serotonergic involvement for the cue produced by entactogens. The third criterion involves the demonstration of differences between the entactogen cue and those produced by other drug classes. This will be discussed in the following sections.

Since data from experiments with MDA were already available in the literature (Glennon and Young, 1984b), efforts were directed toward training two groups of rats to discriminate (±)-MDMA from saline and (+)-MBDB from saline. The discrimination of (+)-MBDB and saline was successfully learned with the initially selected training dose of 1.75 mg/kg of the hydrochloride salt. This dose was selected on the basis of previously conducted substitution tests in MDMA-trained rats (Oberlender and Nichols, 1988). The number of sessions to criterion for this group was 31 ± 10 (mean ± SE).

Initial attempts to train on a dose of 1.25 mg/kg of MDMA·HCl resulted in a low average accuracy and a prolonged mean number of sessions to criterion of 57 ± 19 (±SE). The training dose was changed to 1.75 mg/kg by a gradual increase of 0.1 mg/kg/week over a 5 week period. The average accuracy improved from 71% to 88% by the end of the fifth week. Two additional weeks of training were given before substitution tests began. Two of the rats in the MDMA group failed to reach the criterion performance and were dropped from the study. Two additional rats from this group died during the experiments. The response rates after both MDMA and (+)-MBDB were not significantly different than after saline ($P < 0.05$, Student's t test).

Cross substitution tests were conducted in each of these groups of rats with the racemic mixtures of MDA, MDMA and MBDB and a control treatment of saline. In addition, stereochemical aspects of the entactogen cue were investigated by testing the optical isomers of each of these compounds, illustrated in Figure 32.



	R_1	R_2	R_3
S-(+)-MDA	CH_3	H	H
R-(-)-MDA	H	CH_3	H
S-(+)-MDMA	CH_3	H	CH_3
R-(-)-MDMA	H	CH_3	CH_3
S-(+)-MBDB	CH_2CH_3	H	CH_3
R-(-)-MBDB	H	CH_2CH_3	CH_3

Figure 32. Molecular structures of entactogen optical isomers.

An efficient asymmetric synthesis was employed, as illustrated in Figure 33, for preparing the enantiomers with optical purities greater than 98% (Nichols et al., 1986b). The results of the DD substitution tests in rats trained to discriminate entactogens from saline are given in Table 9.

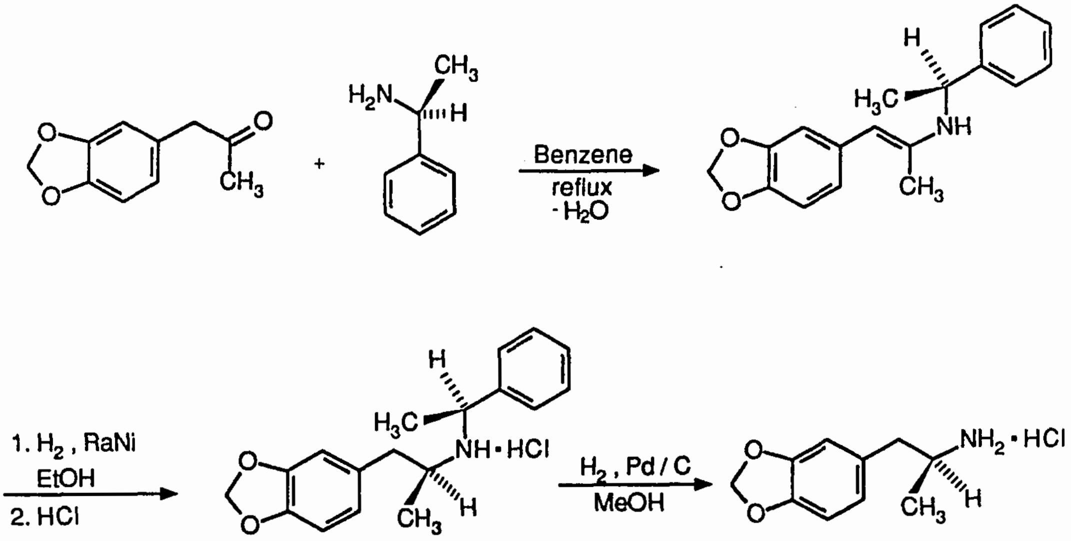


Figure 33. Asymmetric synthesis of *S*-(+)-MDA.

Table 9. Results of DD cross-substitution tests in rats trained to discriminate entactogens from saline.

	ED_{50} (µmol/kg) and 95% C.I. in rats trained to discriminate saline from:	
DRUG	MDMA	(+)-MBDB
(±)-MDA	4.06 (2.59-6.38)	2.09 (1.36-3.21)
S-(+)-MDA	1.63 (0.94-2.81)	1.43 (0.90-2.29)
R-(-)-MDA	2.27 (1.37-3.77)	3.09 (1.88-5.07)
(±)-MDMA	3.40 (2.25-5.13)	3.35 (2.35-4.77)
S-(+)-MDMA	1.92 (1.21-3.06)	1.67 (0.98-2.86)
R-(-)-MDMA	5.03 (3.48-7.29)	3.09 (1.80-5.32)
(±)-MBDB	4.19 (3.03-5.79)	2.92 (2.17-3.92)
S-(+)-MBDB	3.67 (2.70-4.99)	3.28 (2.15-5.01)
R-(-)-MBDB	6.71 (5.16-8.73)	6.51 (4.54-9.34)
Saline	All rats tested selected the saline lever.	

The data presented in Table 9 provide strong evidence that MDA, MDMA and MBDB share a common activity. All of the treatments completely substituted for both MDMA and (+)-MBDB except saline. None of the compounds produced significant disruptive effects (see Tables 17 and 18, appendix B). MDMA has been previously observed to substitute for MDA (1.5 mg/kg) at nondisruptive doses when the latter was employed as the training stimulus (Glennon and Young, 1984b). These results, combined with data gathered elsewhere, allow certain conclusions to be made with respect to entactogen SARs.

Activity is retained when the primary amine, MDA, is *N*-alkylated. This is apparent from the results in Table 9, since both MDMA and MBDB are *N*-methyl derivatives. In addition, Boja and Schechter (1987) have trained rats to discriminate *N*-ethyl MDA (MDE) from saline and showed that its DS properties were similar to those of MDMA. The potency of these derivatives decreases as the substituent changes from hydrogen to methyl to ethyl (Table 9; Boja and Schechter, 1987; Braun et al., 1980). *N*,*N*-dimethylation eliminates central activity (Shulgin, 1981).

Studies with a large series of *N*-substituted MDA analogues have demonstrated that as the bulk of the *N*-substituent increases, attenuation of both the central activity (Braun et al., 1980) and toxicity (Noggle et al., 1987) is observed. The *N*-OH derivative of MDA reportedly has MDA-like activity in humans at comparable doses (Braun et al., 1980). Consideration should be given, however, to the possibility that *N*-OH-MDA is acting as a prodrug. The metabolic conversion of other *N*-OH amphetamine derivatives to the primary amines has been demonstrated (e.g. Fuller et al., 1974).

As is the case with other PEA derivatives, the α-alkyl group of entactogens seems to be important. The toxicity of methylenedioxy-PEA is less than that of MDA (Hardman et al., 1973) and it seems to lack activity in rats (Glennon et al., 1988). The *N*-methyl derivative of this compound was tested clinically as an antitussive and was found to be effective at a dose of 30 mg with no mention of central side-effects (Shulgin, 1978).

The α-ethyl group of MBDB seems to attenuate entactogen activity only slightly. It does, however, dramatically affect other aspects of drug action observed with MDA and MDMA. The evidence for this serves as an important argument for the unique activity of entactogens and will be discussed later. Derivatives of MDA with alkyl groups larger than ethyl have not yet been evaluated for entactogen activity. The α,α-dimethyl analogues of MDA and MDMA have been prepared. The latter compound lacked MDMA-like activity *in vitro* (Nichols et al., 1982) and in man (reference in Nichols and Oberlender, in press).

Further evidence that MDA, MDMA, and MBDB exert similar activity is provided by the results obtained with the stereoisomers (Table 9). The *S*-(+)-isomers of all three entactogens were found to be more potent than their antipodes. In rats trained to discriminate (±)-MDA from saline, substitution tests with (+)-MDA and (-)-MDA demonstrated a similar stereoselectivity (Glennon and Young 1984b). The observed stereoselectivity for the behavioral activity of MBDB parallels that observed in a clinical evaluation (Nichols et al. 1986b). The greater potency of the *S*-(+)-isomer of MDMA, compared to (±)- and *R*-(-)-MDMA, is in agreement with a behavioral study involving schedule-controlled responding in mice (Glennon et al. 1987) and with a clinical report (Anderson et al. 1978). Schechter (1987) has recently reported a similar stereoselectivity for MDMA (*S* > *R*), although in that DD study, (±)-MDMA was more potent than either isomer.

The four compounds shown in Figure 34 represent rigid analogues of two MDA conformations, A and B. Rotation about the benzylic bond of MDA allows A and B to interchange, but only one should approximate the active binding conformation of the molecule. As indicated in Table 10 (for data, see Table 19, appendix B), 2-amino-5,6-methylenedioxyindan (5,6-MDAI) was equipotent with MDMA while 2-amino-6,7-methylenedioxytetralin (6,7-MDAT) was about half as potent. Conformation A, therefore, may best approximate the bioactive conformation.

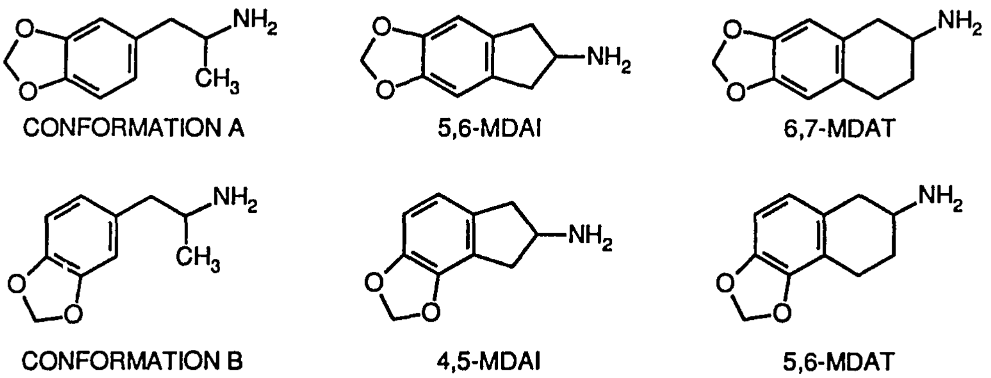


Figure 34. Molecular structures of aminoindan and aminotetralin analogues of MDA.

Table 10. Results of substitution testing with aminoindan and aminotetralin analogues of MDA in MDMA-trained rats.

TEST DRUG	RESULT-ED_{50} in µmol/kg (95% C.I.)
MDMA	Complete Substitution - 2.79 (2.00-3.87)
Saline	All rats selected saline lever
5,6-MDAI	Complete Substitution - 2.75 (1.61-4.69)
6,7-MDAT	Complete Substitution - 5.68 (3.31-9.73)
4,5-MDAI	Partial Substitution
5,6-MDAT	Partial Substitution

The lack of complete substitution for MDMA by 2-amino-4,5-methylenedioxyindan (4,5-MDAI) and 2-amino-5,6-methylenedioxytetralin (5,6-MDAT) may indicate that the methylenedioxy-substituent has been locked into a position, relative to the side chain, that resembles less closely that of entactogens as they bind. Related to this, 2,3-MDA completely substitutes for 3,4-MDA but retains only about one fifth the potency of its positional isomer (Glennon et al., 1984). The 3,4-aromatic substitution pattern, characteristic of entactogens, is quite unusual except for catecholamines. Metabolism of MDA to α-methyldopamine occurs in the rat and may be involved in the observed activity and/or toxicity (Marquardt et al., 1978).

The majority of evidence indicates that entactogens may exert their actions by causing the release of serotonin from serotonin nerve terminals in the brain (Nichols, 1986). Although noradrenergic effects may also be involved in the primary activity, dopaminergic effects seem to exert only a secondary, noncritical influence. Results from DD experiments support this idea. In substitution tests, it was found that the serotonin releasing agent fenfluramine (White and Appel, 1981; Fuller et al., 1988) substitutes for (+)-MBDB at a relatively low dose accompanied by very few disruptive effects. The ED_{50} and 95% CI were found to be 2.01 μmol/kg (1.30-3.09). The complete substitution of MDMA for fenfluramine (Schechter, 1986) further supports the view that serotonin release may be involved in the actions of entactogens.

When rats trained to discriminate (+)-MBDB from saline were pretreated with the serotonin antagonist metergoline, then given the training dose of (+)-MBDB, a dose dependent decrease in drug-appropriate resonding was observed (see Table 11). The number of rats selecting the drug lever decreased from 100% for (+)-MBDB alone to 25% when pretreated with a 0.5 mg/kg dose of metergoline. These results suggest that a class of serotonin receptors may be involved in the production of the (+)-MBDB cue.

By contrast, when the dopamine antagonist haloperidol was administered in the same manner, the training cue was still perceived by the rats. An identical procedure using haloperidol (0.3 mg/kg) as a pretreatment in (+)-amphetamine-trained rats produced a complete block of the training cue. This result is in agreement with previously published work and supports the concept of dopamine-mediation in the stimulant cue (Colpaert et al., 1978). More importantly, these data are inconsistent with a critical involvement of dopamine in the discriminative stimulus properties of entactogens.

Table 11. Results of antagonist tests in (+)-MBDB-trained rats.

TREATMENT	ANTAG. DOSE[a]	TIME[b]	N[c]	%D[d]	%SDL[e]
Metergoline	0	-	8	0	100
followed by	0.125	90	11	27	88
(+)-MBDB	0.25	90	11	27	63
(1.75 mg/kg)	0.5	90	12	25	25
Haloperidol	0	-	8	0	100
followed by	0.05	45	9	11	100
(+)-MBDB	0.1	45	11	36	100
(1.75 mg/kg)	0.25	45	10	20	88
	0.5	45	6	83	D[f]

[a] Dose expressed in mg/kg.
[b] Time = minutes before test session; (+)-MBDB given at 30 min.
[c] N = number of rats tested.
[d] %D = percentage of N rats disrupted.
[e] %SDL = percentage of non-disrupted rats selecting the drug lever.
[f] D = dose which produces disruption of bar pressing.

Additional support for the important role of presynaptic serotonin mechanisms in the actions of entactogens is provided by *in vitro* studies. MDA, MDMA, and MBDB have all been shown to release [^{3}H]-5-HT (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987) and block its reuptake (Steele et al., 1987; Schmidt, 1987) *in vitro* from rat brain synaptosomes. This uptake inhibition may be related to interactions with the serotonin transporter complex since, in binding experiments, MDMA apparently has an affinity for the serotonin uptake site that is nearly an order of manitude higher than any of the 19 other sites studied (Battaglia et al., 1988). There is little evidence to date to suggest that entactogen activity involves direct postsynaptic receptor interactions.

The amphetamine derivatives, MDA and MDMA also affect dopaminergic systems (Johnson et al., 1986; Stone et al., 1986; Steele et al., 1987; Schmidt, 1987) but significantly higher doses are required, relative to serotonergic systems. In addition, the net activity of these compounds on dopaminergic systems may be influenced by an inhibitory effect resulting from the more potent serotonergic activation (Moore, 1978). In a recent study, Gold and Koob (1988) observed an increase in spontaneous motor activity in MDMA-treated rats, when the serotonin antagonist methysergide was administered as a pretreatment. By contrast, methysergide did not potentiate the locomotor hyperactivity produced by amphetamine. Thus, the stimulant component observed in the pharmacolgical profiles of MDA and MDMA, but not amphetamine, seems to be modulated by serotonergic influences.

The most important evidence concerning the relative roles of serotonin and dopamine in the behavioral effects of entactogens comes from *in vitro* experiments with MBDB. The α-ethyl substituent apparently abolishes dopaminergic activity, while only slightly attenuating serotonergic activity, as measured in release (Johnson et al., 1986) and uptake inhibition (Steele et al., 1987) experiments. MBDB-induced dopamine release has been observed in PC-12 cells grown in culture (Monti et al., 1988) but it is not clear how the results in this type of system relate to behavioral effects in intact animals. The implications of these observations with respect to entactogens as a distinct drug class will be discussed later.

Additional work is needed in order to refine our understanding of the mechanism of action of entactogens. For example, although fenfluramine possesses discriminative stimulus properties similar to entactogens, it produces distinctly different effects in humans (Woodward, 1970; Griffith et al., 1975). It seems likely, therefore, that serotonin release alone cannot account for the complete neurochemical mediation of entactogen pharmacology.

Data from *in vitro* studies (Steele et al., 1987) suggest a potential role for norepinephrine (NE). Preliminary experiments were performed with (-)-tomoxetine, a selective NE uptake inhibitor (Wong et al., 1982). Substitution tests in MDMA-trained rats resulted in partial generalization to this compound (67% of the responding rats selected the drug lever after 5 mg/kg), indicating that some overlap in pharmacology may exist. Other neurotransmitter systems may also be involved.

Entactogens and Hallucinogens

The results of initial studies (Nichols et al., 1986) generally demonstrated the lack of LSD-like discriminative stimulus properties for the members of the entactogen drug class. This was confirmed and extended to other hallucinogens in tests with rats trained on entactogens. These results are summarized in Table 12.

Table 12. Results of DD tests comparing entactogens and hallucinogens.

	-----------RESULT (DEGREE OF SUBSTITUTION)-----------		
TRAINING DRUG (dose)[a]	COMPLETE[b]	PARTIAL	NONE
LSD (0.186)	(±)-MDA 4.52 (3.11-6.57) (-)-MDA 2.94 (1.67-5.17)		(+)-MDA (±),(+),(-)-MDMA (±),(+),(-)-MBDB
(±)-MDMA (7.63)		LSD	DOM
(+)-MBDB (7.19)			LSD, DOM, Mescaline

[a] expressed in terms of μmol/kg.

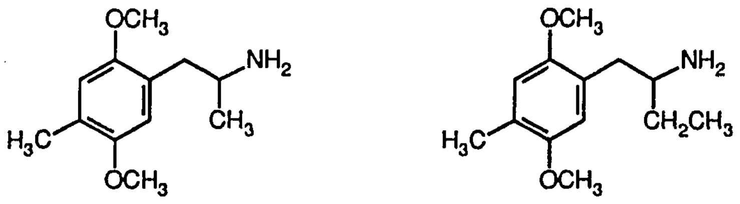
[b] ED_{50} and 95% Confidence interval (μmol/kg)

It seems clear that entactogen activity is distinct from that of hallucinogens. The evidence for this is based on three distinct SAR differences between the two drug classes. The most compelling of these is the effect of an α-ethyl substituent, as in MBDB. The lack of similarity of the DS properties of this compound and those of hallucinogens is apparent in the cross substitution test results.

Both of the isomers of MBDB as well as the racemate failed to substitute for LSD (data in Table 20, appendix B). None of the doses tested resulted in greater than 40% of the rats selecting the drug lever. In (+)-MBDB-trained rats, LSD, DOM, and mescaline all failed to substitute for the training drug (data in Table 22, appendix B). A maximum of 57% of the rats selected the (+)-MBDB lever after 0.16 mg/kg of LSD, a dose which disrupted more than half of the rats tested. DOM produced a maximum of 50% (0.5 mg/kg, 27% disrupted) while the maximum for mescaline was only 43% (10 mg/kg, 50% disrupted).

These results are consistent with the established SAR for hallucinogenic phenethylamines (Nichols, 1981). For example, the prototypical PEA hallucinogen, DOM, seems to lose its characteristic activity when its methyl group is replaced by an ethyl group (Standridge et al., 1976), as in BL 3912 (see Figure 31). Doses of the *R*-(-)-isomer of this compound, BL-3912A, that were more than 100 times greater than those at which DOM is effective did not produce hallucinogenic activity in clinical studies (Winter 1980).

The second SAR difference between entactogens and hallucinogens involves methylation of the nitrogen atom. This molecular modification is known to attenuate the activity of hallucinogenic phenethylamines (Shulgin, 1978). The results of the present experiments are consistent with this since the DS properties of MDMA were found to be unlike hallucinogens. In LSD-trained rats, no substitution occurred with (±)-, (+)-, or (-)-MDMA (data in Table 20, appendix B). Similar results were reported when DOM was used as the training drug (Glennon et al., 1982). In MDMA-trained rats DOM did not substitute: a maximium of 56% of the rats selecting the MDMA lever at a dose of 0.5 mg/kg.



DOM BL-3912

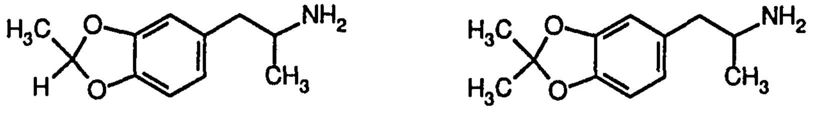
Figure 35. Molecular structures of DOM and BL-3912.

Substitution tests with LSD in MDMA trained rats yielded results that are more difficult to interpret (data in Table 21, appendix B). LSD produced partial substitution with a maximum of 78% of the rats selecting the MDMA lever, at a dose (0.16 mg/kg) which disrupted more than one-third of the rats tested. These data suggest that differences between the subjective effects of LSD and MDMA are more apparent to rats trained with LSD than rats trained with MDMA. If LSD has an MDMA-like component, it is interesting that no substitution of LSD for (+)-MBDB was observed. This suggests that such a component may be unrelated to the common effects of entactogens.

Certain compounds can apparently exert entactogen-like and hallucinogen-like effects. This seems to be the case with (-)-MDA and (±)-MDA which completely substitute for MDMA and (+)-MBDB, as well as for LSD (see data in Table 20, appendix B). These results are in agreement with other reports in the literature. Rats trained to recognize (±)-MDA responded predominantly on the drug lever when tested with LSD (Glennon and Young, 1984c) and both (±)-MDA and (-)-MDA completely substitute for the hallucinogenic training drugs DOM (Glennon et al., 1982) and 5-MeO-DMT (Glennon et al., 1981).

Interestingly, rats trained with LSD, DOM, and 5-MeO-DMT do not recognize (+)-MDA as similar to the training drug. These data provide evidence for the third SAR difference between entactogens and hallucinogens, stereoselectivity. Whereas the stereoselectivity of entactogens is *S* > *R*, as discussed in the previous section, it is the *R* isomers of hallucinogenic amphetamines that are more potent (Nichols and Glennon, 1984). Thus, *S*-(+)-MDA is clearly more potent than the *R*-(-)-MDA as an entactogen, yet is inactive as an hallucinogen.

Two other compounds have also been observed to produce both entactogen and hallucinogen activities in DD tests. These are the dioxole ring-alkylated MDA analogues 3,4-ethylidenedioxyamphetamine (EDA) and 3,4-isopropylidenedioxyamphetamine (IDA), illustrated in Figure 36. These compounds were synthesized and evaluated in order to determine the effects on activity of steric bulk on the dioxole ring (Nichols et al., 1977).



EDA IDA

Figure 36. Molecular structures of EDA and IDA.

The results of substitution testing of these compounds in LSD-(data in Table 23, appendix B) and MDMA-trained rats (data in Table 24, appendix B) are summarized in Table 13. Both compounds completely substituted for both training drugs indicating that, like the parent compound MDA, these dioxole ring alkylated analogues can elicit hallucinogen- and entactogen- like effects. The results from Table 13 also demonstrate the deleterious effect of steric bulk on both activities. The ED_{50} values for MDA are nearly equivalent between the two groups. The potency of MDA decreases upon addition of the first methyl group to the dioxole methylene (EDA) and decreases again when the second methyl group is present (IDA). A trend is observable in which LSD-like activity seems to be more sensitive to the effects of the additional bulk than does MDMA-like activity. Thus, MDA, EDA, and IDA may produce hallucinogen and entactogen actions simultaneously but through different mechanisms.

Table 13. Results of DD substitution testing with EDA and IDA.

TEST	ED_{50} in μmol/kg (95% CI) in rats trained with:	
DRUG	LSD	MDMA
MDA	4.52 (3.11-6.57)	4.06 (2.59-6.38)
EDA	13.39 (7.13-25.12)	8.09 (4.28-15.31)
IDA	29.25 (14.75-57.99)	21.41 (12.51-36.66)

There is evidence reflecting the different mechanisms by which entactogens and hallucinogens produce their stimulus effects. As previously discussed, the neurochemical events mediating the entactogen cue seem to involve presynaptic serotonergic actions. By contrast, a variety of experimental data suggest that the stimulus properties of DOM are related to postsynpatic agonist activity at 5-HT_2 receptors (Glennon et al., 1986). Similar pharmacological properties may mediate the stimulus properties of LSD in rats (Appel and Cunningham, 1986), as discussed in Chapter 1.

The majority of evidence seems to indicate that 5-HT_2 receptors are not similarly involved in the stimulus properties of entactogens. The affinity of MDMA for these receptors is relatively low and the more active *S* isomer has lower affinity than the *R* isomer (Lyon et al., 1986). However, the (+)-MBDB cue was partially blocked with the selective 5-HT_2 antagonist ketanserin. The maximum antagonist effect was seen after a pretreatment dose of 2 mg/kg. Out of 9 rats given that dose followed by the training dose of (+)-MBDB, 4 selected the drug lever (44%). The next higher dose of ketanserin tested, 3 mg/kg, resulted in 8/9 rats selecting the drug lever (89%).

By contrast, the antagonism of hallucinogen cues by ketanserin is complete and follows a normal dose-response relationship typical of competitive antagonism. The 5-HT_2 antagonist pirenpirone, which completely blocked the LSD cue in rats (Colpaert et al., 1982), was only effective in reversing the disruption of operant responding in mice caused by (-)-MDA but not (+)-MDA, (+)-MDMA or (-)-MDMA (Rosecrans and Glennon, 1987). This suggests that, in mice, the 5-HT_2 receptor is important in the hallucinogen-like behavioral properties of (-)-MDA, but may be less critical in mediating the effects of (+)-MDA and the isomers of MDMA.

Clearly, additional work is needed in order to determine which subtypes of serotonin receptors are critical to the actions of entactogens. If the 5-HT_2 receptor is involved, it probably does not have the key role that it seems to have in the actions of hallucinogens. The difference may relate to the **indirect** serotonin agonist properties of entactogens verses the **direct** agonist actions of hallucinogens.

In conclusion, the evidence supports the concept that hallucinogens and entactogens produce distinct effects. This can be seen in terms of the contrasting effects on these activities relating to N-methylation, α-alkyl extension from methyl to ethyl, and stereoselectivity. Aside from compounds such as MDA, EDA, and IDA, which exert both types of actions, there are no examples of hallucinogenic PEA derivatives with only a 3,4-aromatic substitution.

Entactogens and Stimulants

The next step in testing the hypothesis that entactogens are a unique drug class involves a comparison with stimulants. Additional experiments, therefore, employed (+)-amphetamine-trained rats. This group rapidly learned the discrimination task with a mean number of sessions to criterion of 31 ± 2 (±SE). Response rates after drug and saline were not significantly different ($p > 0.05$, Student's t test). The results of substitution tests with stimulants in rats trained to discriminate saline from (+)-amphetamine, MDMA, and (+)-MBDB are presented in Table 14.

Table 14. Results of DD substitution studies comparing entactogens and stimulants.

TRAINING DRUG (DOSE, μM/kg)	RESULT (DEGREE OF SUBSTITUTION):		
	COMPLETE[a]	PARTIAL	NONE
(+)-Amp (5.46)	(+)-Amp 1.69 (1.23-2.31) (+)-Methamp 1.90 (1.20-2.99) MPB[b] 19.62 (13.47-28.57)		(±),(+),(-)-MDA (±),(+),(-)-MDMA (±),(+),(-)-MBDB
MDMA (7.63)	(+)-Amp 4.22 (2.38-7.49) Cocaine 13.8 (9.25-20.43)		
(+)-MBDB (7.19)		Cocaine	(+)-AMP (+)-Methamp

[a] ED_{50} (95% C.I.) expressed in terms of μmol/kg.
[b] MPB = 2-methylamino-1-phenylbutane, the α-ethyl homologue of methamp.

In the (+)-amphetamine-trained group, the training drug produced a smooth dose-response curve (see data in Table 26, appendix B) with an ED_{50} that is very close to the value obtained in an earlier study (Kuhn et al., 1974). None of the rats tested with saline selected the drug lever. In agreement with previous work (Young and Glennon, 1986), (+)-methamphetamine completely substituted for this training drug. The α-ethyl homologue of methamphetamine, MPB, also completely substituted with an ED_{50} reflecting a potency an order of magnitude lower than the α-methyl derivative. None of the entactogens were perceived as similar to (+)-amphetamine (see data in Table 26, appendix B).

In the entactogen-trained rats, (+)-amphetamine and cocaine were both recognized as MDMA-like. Complete substitution of (+)-amphetamine was accompanied by disruption in 7 of the 13 rats tested (see data in Table 25, appendix B). Different results were obtained in the (+)-MBDB-trained rats (see data in Table 27, appendix B). Cocaine produced a maximum of 63% drug-appropriate responding while a maximum of 50% was observed for all doses of (+)-amphetamine and (+)-methamphetamine.

These results support the hypothesis that entactogen and stimulant pharmacology is not identical. The prototype entactogen, MBDB, and the prototype stimulant, (+)-amphetamine, were found to be clearly distinguishable from each other when cross tested. This supports the view that the psychopharmacology of entactogens is different from stimulant activity and may reflect the apparent lack of significant dopaminergic activity of MBDB (Johnson et al., 1986; Steele et al., 1987).

Since (+)-amphetamine has very weak effects on serotonergic systems (Moore, 1978), its DS properties are easily distinguishable from those of drugs which do not share, to some extent, common effects involving dopamine. This view is supported by results from DD studies employing fenfluramine as a training drug, since (+)-amphetamine consistently fails to produce substitution (Goudie, 1977; White and Appel, 1981; McElroy and Feldman, 1984). Furthermore, the dopamine antagonist haloperidol does not affect the fenfluramine cue (White and Appel, 1981), or the (+)-MBDB cue, as discussed earlier (Table 11).

Interestingly, although the stimulant cocaine produces DS effects mediated primarily through dopaminergic mechanisms (Colpaert et al., 1978), secondary effects on serotonergic systems have been discussed in relation to its partial substitution in fenfluramine-trained rats (White and Appel, 1981). The same serotonergic effects of cocaine may also account for the partial substitution for (+)-MBDB, and the decreased disruptive effects, relative to (+)-amphetamine, observed in its complete substitution for MDMA. These results are in agreement with those of Broadbent et al. (1987) and Glennon and Young (1984c) in which similarities were observed between cocaine and MDA.

In view of the secondary effects of MDA and MDMA on dopamine uptake and release (Johnson et al. 1986; Stone et al. 1986; Steele et al. 1987), it seems reasonable that they share some DS properties with cocaine and (+)-amphetamine. However, the results of *in vitro* studies in rat striatum (Schmidt et al. 1987) indicate that MDA and MDMA are both an order of magnitude more potent in releasing [^{3}H]-serotonin than [^{3}H]-dopamine. This difference may account for the large numbers of disruptions which accompanied the complete substitution of MDMA, and MDA for (+)-amphetamine (Glennon and Young, 1984a).

If entactogens are simply acting as stimulants, one would not expect to see the high ED_{50} values obtained for (+)-amphetamine in both MDMA- and MDA-trained rats (Table 12; Glennon and Young, 1984a). Assays of activity in man and animals indicate that amphetamine is about 10 times more potent than MDA or MDMA. Yet, the results from DD experiments, employing entactogens as training drugs, indicate that amphetamine is less potent than MDA and MDMA. This suggests that the primary discriminative cue of MDMA cannot simply be "amphetamine-like."

The lack of substitution observed for the racemates and optical isomers of entactogens in (+)-amphetamine-trained rats provides further support for this. Even at high doses of MDA and MDMA, rats trained on (+)-amphetamine failed to recognize the effects of these test drugs as similar to those of their training drug. In order to test the possiblity that stimulant-like effects might become apparent sometime after the 30 minute interval used between injections and testing, several doses of MDMA and (+)-MDA were tested using 60 and 90 minute pretreatment intervals. No substitution was observed at either of these times (see Table 28, appendix B).

These results are consistent with those reported in several similar studies in the literature (Shannon, 1980; Broadbent et al., 1987), but not in others (Glennon and Young 1984b; Evans and Johanson 1986; Kamien et al. 1986). Procedural differences such as the schedule of reinforcement, the reward used, and numbers of animals tested may account for the discrepancy. Particularly noteworthy is the small number of animals used in the studies which reported complete substitution of MDA and MDMA for (+)-amphetamine.

Despite the lack of consistency in the results of this particular aspect of these studies, certain SAR differences support the hypothesis that entactogen and stimulant activities are distinct. For example, in the last section, the stereospecific hallucinogenic activity of *R*-(-)-MDA was discussed. Similarly, in studies where MDA and MDMA have been observed to substitute for (+)-amphetamine, a stereospecific effect of the *S*-(+)-isomers is observed; no stimulant-like component has been observed for the *R*-(-)-isomers (Glennon and Young, 1984b; Glennon et al., 1988).

The net effect of (±)-MDA can therefore be viewed as the result of the simultaneous actions and interactions of two different drugs, which happen to be enantiomers. While both isomers produce entactogen activity (Table 9), only the (-)-enantiomer produces hallucinogen-like activity, while the stimulant-like activity, observed in certain studies, seems to be due to (+)-MDA.

The result of N-methylation of MDA can be interpreted as abolishing the hallucinogen-like activity of (-)-MDA (Table 12), but only slightly attenuating the entactogen activities of both isomers (Table 9; Glennon and Young, 1984b). The "(+)-amphetamine-like" activity of MDA (Glennon and Young, 1984a), seemed to be increased with N-methylation since, in (+)-amphetamine-trained rats, (+)-MDMA was found to be more potent than (+)-MDA (Glennon and Young, 1984b; Glennon et al., 1988). Thus, these differences for the effect of N-methylation of MDA may reflect the distinctive binding sites for each activity.

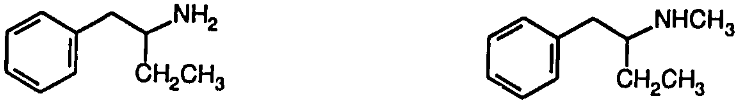
This conclusion is supported by previous analyses of stereochemical aspects of drug actions. Ariëns (1987) has noted that if a compound has multiple pharmacological actions, and if the eudismic ratios (activity of the more active stereoisomer/activity of the less active stereoisomer) differ for the different effects, this indicates that the effects are based on different mechanisms, involving different receptors. With this in mind, the following argument relates to the primary effects of entactogens as distinct from other, occasionally observed, secondary effects.

As discussed previously , the *stereoselective* action (*S* > *R*) of entactogens has been demonstrated consistently. Similar stereoselectivity is observed for the stimulant activity of amphetamine and related compounds such as cathinone (Glennon et al., 1984). However, the "amphetamine-like" activity of both MDA and MDMA, in cases where it has been observed, is stereo*specific* rather than stereo*selective*. The same is true for the hallucinogen-like activity of MDA. Thus, considering the general relationship described by Ariëns, entactogen activity may be unique.

Furthermore, MDE, the N-ethyl derivative of MDA shares stimulus properties with MDMA (Boja and Schechter, 1987), and N-OH-MDA is reportedly similar in its actions to MDA (Braun et al., 1980). Yet, neither substitutes for (+)-amphetamine under conditions identical to those used when complete substitution of MDA and MDMA for (+)-amphetamine was reported (Glennon et al., 1988). By contrast, the N-ethyl and N-OH derivatives of amphetamine completely substitute for (+)-amphetamine (Glennon et al., 1988). Thus, either an ethyl or hydroxy substituent on the nitrogen abolishes the "amphetamine-like" effects of MDA but not of amphetamine itself. This difference in structure-activity relationships lends further support to the concept of different mechanisms for entactogen and stimulant activities.

Differences between the effects of MDMA and (+)-amphetamine have also been observed when compared in an unpunished, schedule-controlled behavioral paradigm in pigeons (Boszormenyi et al., 1986). Considering all of the results discussed above, it seems likely that amphetamine-like activity is not equivalent to entactogen-like activity. The most important evidence for this seems to be the attenuation of the effects that MDA and MDMA may share with stimulants, by extending the α-methyl to an α-ethyl. This important finding stimulated our interest in understanding the effect of an α-ethyl group on stimulant activity.

The few studies that were found in the literature suggest that stimulant activity in PEAs is generally reduced by alkyl groups larger than methyl at the α-position. Early work by van der Schoot et al. (1962) reported that the α-ethyl homologue of amphetamine, 2-amino-1-phenylbutane (see Figure 37), had only 16% the potency of amphetamine in stimulating spontaneous motor activity in mice. However, this compound was patented as a stimulant (Ullyot, 1948).

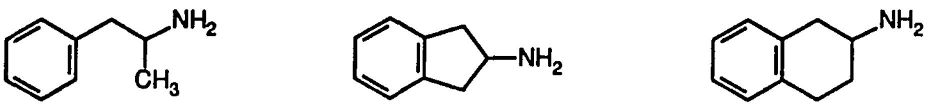


2-AMINO-1-PHENYLBUTANE 2-METHYLAMINO-1-PHENYLBUTANE

Figure 37. Molecular structures of the α-ethyl homologues of amphetamine and methamphetamine.

was synthesized and compared with (+)-methamphetamine in rats trained to discriminate (+)-amphetamine from saline. The racemic α-ethyl compound was an order of magnitude less potent than (+)-methamphetamine (see Table 14). This can be compared with the two-fold decrease in potency for (±)-MBDB relative to (+)-MDMA in MDMA-trained rats (Table 9). It seems reasonable to conclude that the presence of an α-ethyl substituent has only a small effect on entactogenic activity compared with stimulant activity.

If the methylenedioxy-substituted series represent true stimulants, the active conformation of their side chains, as reflected in the activities of rigid analogues, should be identical to that of amphetamine. The aminoindan and tetralin analogues of amphetamine, shown in Figure 38, have been tested in (+)-amphetamine-trained rats (Glennon et al., 1984) and the results obtained differ significantly with those obtained for the methylenedioxy-substituted analogues. As described earlier, the indan derivative, 5,6-MDAI was equipotent to MDMA and twice as active as the tetralin derivative, 6,7-MDAT (see Figure 34). By contrast, it was reported that the ring-unsubstituted tetralin, 2-AT, was closer in potency to amphetamine, while 2-AI was only half as potent as 2-AT (Glennon et al., 1984).



AMPHETAMINE 2-AMINOINDAN (2-AI) 2-AMINOTETRALIN (2-AT)

Figure 38. Molecular structures of 2-aminoindan and 2-aminotetralin.

The reason for the difference in potency of 2-AI and 2-AT was suggested to relate to the degree to which the rigid analogues could mimic the conformation of amphetamine necessary for producing its characteristic stimulus effects

(Glennon et al., 1984). Therefore, the results of testing these analogous derivatives in MDMA- and (+)-amphetamine-trained rats suggest that different active conformations are preferred for the side chains of stimulants and entactogens, implying different sites of action.

Second Generation Entactogens

Part of the effort in DD studies with entactogens has been directed toward identifying compounds that may eventually become clinically useful agents. A discussion of the value to psychiatry, associated with the development of such agents, was presented in Chapter 1. In practical terms, the goal of this aspect of the work was to identify compounds which retain the DS properties of MDMA and MBDB, but do not share common effects with hallucinogens and stimulants. A good example of such a drug is one of the compounds discussed in the previous section, 5,6-methylenedioxy-2-aminoindan (5,6-MDAI, Figure 34).

The results obtained with 5,6-MDAI illustrate an ideal behavioral profile for a second generation entactogen (see Table 29, appendix B). Complete substitution was observed for MDMA (Table 10) and (+)-MBDB (ED_{50} = 2.04 μM/kg, 95% C.I. = 1.16-3.57) at low doses with little behavioral disruption. No substitution occurred for LSD and the results of testing in (+)-amphetamine-trained rats were especially noteworthy in that not a single rat selected the drug lever. This complete lack of a stimulant component for 5,6-MDAI may be an important factor because recent theories of addiction describe psychomotor activation, mediated in part by dopaminergic fibers, as a common denominator of a wide range of addictive substances (Wise and Bozarth, 1987). Compounds such as 5,6-MDAI which do not cause psychomotor activation, may be less likely to possess dependence liablility. This factor would probably increase the likelihood of these compounds gaining acceptance as therapeutic tools.

as 5,6-MDAI which do not cause psychomotor activation, may be less likely to possess dependence liablility. This factor would probably increase the likelihood of these compounds gaining acceptance as therapeutic tools.

Another encouraging factor to emerge from studies of 5,6-MDAI is its apparent lack of neurotoxic effects. While MBDB has been found to produce neurotoxicity similar to that seen with MDMA (Johnson and Nichols, 1989), 5,6-MDAI does not produce this effect (Nichols et al., manuscript in preparation). These results reflect positively on the chances of developing new entactogens that can eventually be tested for clinical value.

Future Studies

In the previous sections of this chapter, experimental results were described which provide evidence for the following conclusions.

1. The entactogens, MDA, MDMA, and MBDB, exert a common primary pharmacology.
2. This common activity is probably mediated by mechanisms involving presynaptic serotonergic sites, with possible contributions from effects on noradrenergic, but probably not dopaminergic pathways.
3. Entactogens represent a novel drug class since numerous SAR differences have been observed between their common activity and the activities of both hallucinogens and stimulants.
4. Non-neurotoxic derivatives, potentially suitable for clinical testing are possible. One such compound, 5,6-MDAI, has already been identified.

One of the important questions that will be pursued in future studies involves the mechanism of action of entactogens. In particular, a more detailed comparison with fenfluramine and related derivatives will be pursued.

A group of rats has recently been trained to discriminate (+)-fenfluramine from saline, and additional experiments with selective serotonin agonists and antagonists are underway.

New experiments designed to shed light on the possible noradrenergic component of entactogen activity are also being planned. Eventually, we hope to gain a better understanding of how entactogens work, and in the process, obtain potentially valuable information regarding the neurochemical mediation of certain emotional states. Additional evidence for the novelty of entactogen activity can be anticipated as those studies progress.

In addition, the early results obtained from experiments with 5,6-MDAI are very encouraging. Further efforts will be directed to the identification of additional compounds as candidates for clinical experiments. The potential benefits such compounds may provide, in terms of both the treatment and understanding of emotional disorders, could be significant.

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APPENDICES

Appendix A - Drug Discrimination Methodology

Subjects

Male Sprague-Dawley rats (Harlan Labs, Indianapolis, IN) weighing 200-240g at the start of the experiment were used. Initially, none of the rats had previously received drugs or behavioral training. They were housed individually with free access to water and given a rationed amount of supplemental feeding (Lab Blox) after experimental sessions so as to maintain them at 85% of their free-feeding weight. The animal facility was maintained at a constant temperature of 22-24°C, with lights on at 6 am and off at 8 pm.

Apparatus

Six standard operant chambers (Coulbourn Instruments) consisted of modular test cages enclosed within sound-attenuated cubicles with fans providing ventilation and background noise. Electronic pellet dispensers and data recording were controlled by solid state logic, interfaced through a Coulbourn Instruments Dynaport to an IBM PC micro- computer, all located in an adjacent room.

Procedure

A two-lever procedure with a fixed ratio (FR) 50 schedule of food reinforcement (Bioserv, 45 mg dustless pellets) was used. To avoid positional preference, half of the rats were trained on drug-L, saline-R while the remainder were trained on drug-R, saline-L. Training sessions lasted 15 min and were conducted at the same time each day, Monday through Saturday. Levers were cleaned with 10% ethanol between subjects in order to avoid olfactory cues (Extance and Goudie 1981).

After initial shaping, drug or saline was administered, by intraperitoneal (IP) injection 30 min prior to sessions, randomly ordered, with neither treatment given for more than three consecutive sessions. Only the stimulus-appropriate lever was present. When stabilized response rates were attained, both levers were presented simultaneously and the rats were required to respond on the stimulus-appropriate lever to receive reinforcement.

The schedule of reinforcement was gradually increased from FR 1 to FR 50. This phase of the training continued until each rat had achieved an accuracy of at least 85% (number of correct presses X 100 / number of total presses before the first reinforcement) for 8 of 10 consecutive sessions. Then, every third session was used as a test period as long as the rat maintained an accuracy of at least 85% on the two preceding maintenance sessions (drug and saline). Test solutions were also administered IP, 30 min prior to the session. Test sessions were run under conditions of extinction, with the rats removed from the operant box immediately after 50 presses were completed on one lever. If 50 presses were not completed in 5 min, the session was ended and scored as a disruption. The data for a test session were discarded if the 85% criterion was not met on the following two maintenance sessions (Colpaert et al. 1982). Treatments were randomized at the beginning of the study.

Data Analysis

The scoring of the data was done in a quantal fashion. The lever on which the rat first emitted 50 responses was scored as the "selected" lever. The percentage of rats selecting the drug lever for each dose of test compounds was then used to determine ED_{50} values with 95% confidence intervals, using the method of Litchfield and Wilcoxon (1949). Recent evidence suggests that lever pressing is a quantal function of stimulus intensity and that presentation of lever selection data in the manner described above best reflects the nature of the paradigm (Harris et al., 1988).

Drugs

The following drugs were obtained form the organizations indicated in the parentheses. The abbreviations used are defined on page ix. S-(+)-amphetamine sulfate (Smith Kline & French), S-(+)-methamphetamine·HCl (Aldrich), DOM·HCl (NIDA), DOMAI, DOMAT (Bristol) and LSD tartrate (NIDA).

The following compounds were synthesized in this laboratory by the methods contained in the reference in parentheses: The racemic mixtures and optical isomers of MDA, MDMA and MBDB (Nichols et al. 1986), 6,7-MDAT, 5,6-MDAT, 5,6-MDAI, and 4,5-MDAI (Nichols et al., manuscript in preparation).

All solutions were prepared by dissolving the compounds in saline at a concentration that allowed the appropriate dose to be given in a volume of 1 ml/kg. The doses refer to the salt forms.

Appendix B - Tables of Drug Discrimination Data

The following pages contain raw data from the two lever drug discrimination experiments. The abbreviations used in the tables are defined as follows:

N = the total number of animals tested.

%D = the percentage of N animals which were disrupted or did not finish 50 presses in the 5 minute test session.

%SDL = the percentage of non-disrupted rats selecting the drug lever.

D = disruption in greater than 80% of animals tested

Table 15. DD Data: lysergic acid amides in LSD-trained rats.

DRUG	DOSE μM/kg	mg/kg	N	%D	%SDL
LSD	0.012	0.005	8	0	13
	0.024	0.01	8	0	13
	0.047	0.02	8	0	38
	0.093	0.04	9	0	89
	0.186	0.08	9	0	100
SALINE	-	-	8	0	0
5	0.047	0.02	9	0	22
	0.093	0.04	10	10	22
	0.14	0.06	12	8	27
	0.164	0.07	11	9	40
	0.186	0.08	16	6	60
	0.279	0.12	13	31	67
	0.301	0.13	16	31	82
17-1	0.006	0.002	8	0	0
	0.011	0.004	8	0	13
	0.022	0.008	11	18	44
	0.044	0.016	9	11	63
	0.088	0.032	8	0	100
17-2	0.088	0.032	9	11	0
	0.177	0.063	9	0	22
	0.353	0.126	14	43	50
	0.707	0.252	12	33	38
	1.414	0.505	9	11	63
	2.472	0.883	10	20	100
17-3	0.044	0.016	8	0	0
	0.088	0.032	7	0	57
	0.177	0.063	7	0	57
	0.353	0.126	9	22	57
	0.620	0.220			
	0.707	0.252			
17-4	0.088	0.032	9	0	0
	0.177	0.063	9	0	22
	0.266	0.095	9	0	33
	0.353	0.126	10	0	40
	0.441	0.158	10	0	60
	0.707	0.252	11	9	70
	0.883	0.316	9	0	89

Table 16. DD Data: 4-alkyl-2,5-DMA analogues in LSD-trained rats.

DRUG	DOSE μM/kg	 mg/kg	N	%D	%SDL
LSD	0.012	0.005	8	0	13
	0.024	0.01	8	0	13
	0.047	0.02	8	0	38
	0.093	0.04	9	0	89
	0.186	0.08	9	0	100
SALINE	-	-	8	0	0
DOM	0.51	0.125	7	0	13
	0.77	0.19	9	0	67
	1.02	0.25	9	0	67
	2.04	0.5	9	11	100
24	0.87	0.25	9	0	11
	1.74	0.5	11	18	56
	3.48	1	13	38	63
	4.35	1.25	11	27	63
	4.79	1.38	12	0	83
25	1.74	0.5	9	11	18
	3.48	1	10	20	60
	6.96	2	8	0	70
	13.91	4	13	23	90
R-25	0.87	0.25	11	9	0
	1.74	0.5	12	0	25
	3.48	1	11	9	50
	5.22	1.5	11	9	80
S-25	1.74	0.5	10	0	0
	3.48	1	11	9	60
	5.22	1.5	11	9	30
	6.96	2	11	9	70
	13.92	4	14	29	90

Table 17. DD data: entactogen derivatives in MDMA-trained rats.

	DOSE				
DRUG	µM/kg	mg/kg	N	%D	%SDL
MDMA	0.95	0.22	8	0	0
	1.91	0.44	8	0	25
	3.82	0.88	8	0	63
	5.72	1.31	9	0	56
	7.63	1.75	9	11	100
SALINE	-	-	8	0	0
R-(-)-MDMA	0.95	0.22	8	0	0
	1.91	0.44	8	0	13
	3.82	0.88	8	0	38
	5.72	1.31	8	0	63
	7.63	1.75	8	0	63
	9.54	2.19	8	0	75
	11.45	2.63	9	0	89
S-(+)-MDMA	0.95	0.22	8	0	25
	1.91	0.44	9	0	44
	3.82	0.88	8	0	75
	5.72	1.31	9	11	88
	7.63	1.75	11	27	100
MBDB	1.91	0.47	8	0	0
	3.82	0.93	8	0	38
	5.72	1.39	8	0	50
	7.63	1.86	8	0	88
	9.54	2.32	8	0	100
R-(-)-MBDB	3.82	0.93	8	0	25
	5.72	1.39	8	0	38
	7.63	1.86	8	0	38
	9.54	2.32	8	0	75
	11.45	2.79	9	11	75
	13.36	3.25	9	11	100
S-(+)-MBDB	1.91	0.47	8	0	13
	3.82	0.93	8	0	25
	5.72	1.39	8	0	88
	7.63	1.86	8	0	100
MDA	1.39	0.3	8	0	13
	2.78	0.6	9	11	25
	5.57	1.2	9	11	50
	8.35	1.8	10	20	100

Table 17, continued.

DRUG	DOSE μM/kg	 mg/kg	N	%D	%SDL
R-(-)- MDA	0.95	0.21	9	11	13
	1.91	0.41	12	25	63
	3.82	0.82	10	20	50
	7.63	1.64	10	20	100
S-(+)- MDA	0.48	0.10	9	11	0
	0.95	0.21	10	10	33
	1.91	0.41	12	25	44
	3.82	0.82	9	11	88

Table 18. DD Data: entactogens in (+)-MBDB-trained rats.

DRUG	DOSE µM/kg	 mg/kg	N	%D	%SDL
S-(+)- MBDB	1.80	0.44	8	0	13
	3.59	0.88	8	0	37
	5.39	1.31	9	11	88
	7.19	1.75	8	0	100
SALINE	-	-	8	0	0
MDA	0.95	0.21	9	11	25
	1.91	0.41	11	27	50
	2.86	0.62	9	11	50
	3.82	0.82	12	33	63
	5.73	1.24	12	33	100
R-(-)- MDA	0.95	0.21	10	20	25
	1.91	0.41	11	27	25
	3.82	0.82	9	11	38
	7.63	1.65	12	33	75
	8.58	1.86	14	43	100
S-(+)- MDA	0.48	0.1	9	11	13
	0.95	0.21	8	10	25
	1.91	0.41	12	33	50
	2.86	0.62	10	20	88
	3.82	0.82	11	27	88
MDMA	1.91	0.44	8	0	13
	2.87	0.66	10	20	50
	3.82	0.88	9	11	63
	7.63	1.75	11	28	88
R-(-)- MDMA	1.91	0.44	10	20	38
	3.82	0.88	9	11	50
	7.63	1.75	8	0	75
	11.45	2.63	8	0	100
S-(+)- MDMA	0.95	0.22	8	0	25
	1.91	0.44	10	20	63
	3.82	0.88	9	11	75
	4.77	1.1	10	20	88
MBDB	1.8	0.44	9	11	13
	2.7	0.66	8	0	50
	3.59	0.88	8	0	63
	5.39	1.31	8	0	88
	7.19	1.75	8	0	100

Table 18, continued.

DRUG	DOSE µM/kg	 mg/kg	N	%D	%SDL
R-(-)-	3.59	0.88	8	0	25
MBDB	7.19	1.75	9	11	38
	10.79	2.63	11	27	75
	14.37	3.5	8	0	100

Table 19. DD data: aminoindans and aminotetralins in MDMA-trained rats.

DRUG	DOSE μM/kg	 mg/kg	N	%D	%SDL
MDMA	0.95	0.22	8	0	0
	1.91	0.44	8	0	25
	3.82	0.88	8	0	75
	7.63	1.75	8	0	100
SALINE	-	-	8	0	0
DOMAI	4.11	1	9	11	13
	8.21	2	10	20	38
	16.43	4	12	42	71
	20.54	5	7	71	D
DOMAT	1.94	0.5	10	20	38
	3.88	1	9	33	67
	7.77	2	9	33	33
	11.65	3	6	83	D
4,5-MDAI	1.17	0.25	3	33	0
	2.34	0.5	4	25	67
	4.68	1	9	22	14
	9.37	2	5	20	25
5,6-MDAI	1.17	0.25	8	0	13
	2.34	0.5	10	20	50
	4.68	1	8	0	63
	9.37	2	10	10	100
5,6-MDAT	1.1	0.25	8	38	0
	2.2	0.5	10	30	71
	2.75	0.625	12	33	63
	3.3	0.75	10	20	63
	4.4	1	9	11	50
	6.6	1.5	9	11	75
	7.7	1.75	12	33	75
	8.8	2	12	42	71
6,7-MDAT	2.2	0.5	9	11	13
	4.4	1	9	11	50
	8.8	2	8	0	63
	17.6	4	11	27	88

Table 20. DD Data: entactogen derivatives in LSD-trained rats.

DRUG	DOSE μM/kg	DOSE mg/kg	N	%D	%SDL
LSD	0.006	0.0025	8	0	0
	0.012	0.005	8	0	50
	0.023	0.01	8	0	50
	0.047	0.02	8	0	63
	0.093	0.04	8	0	75
	0.186	0.08	8	0	100
SALINE	-	-	8	0	0
MDA	1.5	0.32	11	3	0
	3	0.63	8	0	25
	6	1.29	10	20	63
	8	1.72	23	65	88
R-(-)- MDA	0.75	0.16	8	0	13
	1.5	0.32	9	11	25
	3	0.63	9	11	25
	6	1.29	10	20	88
S-(+)- MDA	0.75	0.16	8	0	13
	1.5	0.32	8	0	25
	3	0.63	10	20	25
	6	1.29	18	56	38
	7.5	1.62	31	76	33
	8	1.73	5	100	D
MDMA	1.5	0.34	10	20	25
	3	0.69	10	20	0
	6	1.38	8	0	25
	8	1.84	21	62	38
	9	2.19	5	80	D
R-(-)- MDMA	0.75	0.17	8	0	13
	1.5	0.34	8	0	38
	3	0.69	9	0	22
	6	1.38	9	11	25
	7.5	1.72	14	43	50
	9	2.07	18	56	38
	10.5	2.41	19	58	38
	12	2.76	14	36	33
	15	3.44	15	47	50
	18	4.14	20	80	D

Table 20, continued.

DRUG	DOSE		N	%D	%SDL
	µM/kg	mg/kg			
S-(+)-MDMA	0.75	0.17	9	1	25
	1.5	0.34	8	0	25
	3	0.69	9	11	50
	6	1.38	14	43	50
	7.5	1.72	30	73	50
	8	1.83	5	100	D
MBDB	1.5	0.37	12	25	0
	3	0.73	10	20	0
	6	1.46	16	50	13
	8	1.95	17	59	14
	9	2.07	5	100	D
R-(-)-MBDB	0.75	0.17	8	0	38
	1.5	0.37	8	0	13
	3	0.73	8	0	13
	6	1.46	9	11	13
	7.5	1.83	10	20	13
	9	2.19	14	43	25
	10.5	2.56	16	50	25
	12	2.92	14	43	38
	15	3.65	7	86	D
S-(+)-MBDB	0.75	0.17	8	0	0
	1.5	0.37	9	11	13
	3	0.73	8	0	25
	6	1.46	8	0	0
	7.5	1.83	15	47	25
	9	2.19	26	62	40
	10.5	2.56	11	64	50
	12	2.92	6	100	D

Table 21. DD Data: Hallucinogens in MDMA-trained rats.

DRUG	DOSE μM/kg	DOSE mg/kg	N	%D	%SDL
DOM	0.51	0.125	12	33	50
	1.02	0.25	11	18	44
	2.04	0.5	10	10	56
	3.06	0.75	12	33	50
	4.04	1.0	12	50	50
	5.06	1.25	13	38	38
	6.08	1.5	5	100	D
LSD	0.047	0.02	10	20	13
	0.093	0.04	8	0	38
	0.186	0.08	13	31	56
	0.372	0.16	14	36	78
	0.558	0.24	13	54	67

Table 22. DD Data: hallucinogens in (+)-MBDB-trained rats.

	DOSE				
DRUG	µM/kg	mg/kg	N	%D	%SDL
LSD	0.047	0.02	8	0	25
	0.093	0.04	8	0	37
	0.186	0.08	12	17	50
	0.261	0.12	13	69	50
	0.372	0.16	15	53	57
DOM	0.51	0.13	10	20	25
	1.02	0.25	9	22	38
	2.04	0.5	11	27	50
	4.07	1	15	33	40
	5.09	1.25	5	100	D
MESCALINE	10.1	2.5	8	0	13
	20.2	5	9	11	38
	40.41	10	14	50	43
	50.51	12.5	6	67	D

Table 23. DD Data: dioxole ring-alkylated MDA deriviatives in LSD-trained rats.

DRUG	DOSE μM/kg	 mg/kg	N	%D	%SDL
LSD	0.006	0.0025	8	0	0
	0.012	0.005	8	0	50
	0.023	0.01	8	0	50
	0.047	0.02	8	0	63
	0.093	0.04	8	0	75
	0.186	0.08	8	0	100
SALINE	-	-	8	0	0
MDA	1.5	0.32	11	27	0
	3	0.63	8	0	25
	6	1.29	10	20	63
	8	1.72	23	15	88
EDA	2.32	0.53	8	0	0
	4.64	1.07	8	0	13
	9.28	2.13	9	11	50
	18.56	4.26	9	11	50
	37.12	8.52	17	53	88
IDA	4.64	1.13	8	0	0
	9.28	2.26	8	0	25
	37.12	9.04	9	0	33
	74.24	18.08	10	20	88

Table 24. DD Data: dioxole ring-alkylated MDA derivatives in MDMA-trained rats.

DRUG	µM/kg	mg/kg	N	%D	%SDL
MDA	1.39	0.3	8	0	13
	2.78	0.6	9	11	25
	5.57	1.2	9	11	50
	8.25	1.8	10	20	100
EDA	2.18	0.5	8	0	13
	4.36	1.0	9	11	50
	8.71	2.0	10	20	50
	17.43	4.0	8	0	63
	26.14	6.0	8	0	75
	34.85	8.0	9	11	88
IDA	4.11	1.0	8	0	13
	12.32	3.0	8	0	13
	36.96	9.0	8	0	63
	49.28	12.0	9	11	88

Table 25. DD Data: Stimulants in MDMA-trained rats.

DRUG	DOSE μM/kg	DOSE mg/kg	N	%D	%SDL
S-(+)- AMP	2.17	0.4	12	25	22
	3.26	0.6	13	38	50
	4.35	0.8	14	43	63
	5.43	1.0	12	33	38
	6.52	1.2	13	54	83
	7.60	1.4	13	54	67
	10.87	2.0	13	62	60
COCAINE	7.36	2.5	9	11	25
	14.71	5	8	0	50
	22.07	7.5	11	27	63
	29.42	10	11	27	88

Table 26: DD Data: Stimulants and Entactogens in (+)-amphetamine-trained rats.

	DOSE				
DRUG	µM/kg	mg/kg	N	%D	%SDL
S-(+)- AMP first time	1.08	0.2	8	0	13
	1.62	0.3	8	0	63
	2.17	0.4	8	0	75
	4.35	0.8	8	0	88
	5.43	1	8	0	100
	10.87	2	9	11	100
S-(+)- AMP second time	0.34	0.063	9	0	0
	0.68	0.125	8	0	13
	1.36	0.25	8	0	25
	2.72	0.5	8	0	88
	5.43	1	8	0	100
SALINE	-	-	8	0	0
S-(+)- MA	0.67	0.125	8	0	0
	1.35	0.25	9	11	38
	2.70	0.5	8	0	63
	5.39	1	8	0	100
MPB	5.01	1	10	20	0
	7.52	1.5	9	0	11
	10.03	2	14	36	33
	20.06	4	14	43	37
	30.09	6	11	27	63
	40.12	8	11	27	88
MDA	0.48	0.10	8	0	0
	0.95	0.21	8	0	13
	1.91	0.41	9	11	13
	3.82	0.82	8	0	50
	5.73	1.23	12	25	38
	7.63	1.65	13	69	D
R-(-)- MDA	0.48	0.10	8	0	0
	0.95	0.21	8	0	13
	1.91	0.41	9	11	13
	3.82	0.82	10	20	13
	7.63	1.65	6	83	D
S-(+)- MDA	0.48	0.10	8	0	0
	0.95	0.21	8	0	25
	1.91	0.41	11	9	0
	3.82	0.82	13	38	25
	4.77	1.03	6	83	D

Table 26, continued.

DRUG	DOSE µM/kg	DOSE mg/kg	N	%D	%SDL
MDMA	3.82	0.88	8	0	13
	7.63	1.75	8	0	25
	9.81	2.25	14	43	25
	11.45	2.63	7	86	D
R-(-)-MDMA	3.82	0.88	8	0	0
	7.63	1.75	8	0	0
	11.45	2.63	14	43	0
	15.26	3.51	6	83	D
S-(+)-MDMA	1.91	0.44	8	0	0
	3.82	0.88	13	46	0
	4.78	1.10	11	27	13
	5.73	1.31	6	83	D
MBDB	3.59	0.88	8	0	0
	7.19	1.75	8	0	25
	8.99	2.19	15	47	37
	10.78	2.63	15	67	0
	14.37	3.50	8	63	0
	21.56	5.25	5	100	D
R-(-)-MBDB	3.59	0.88	10	20	13
	7.19	1.75	11	27	0
	14.37	3.50	10	20	13
	21.56	5.25	5	100	D
S-(+)-MBDB	1.80	0.44	9	11	0
	3.59	0.88	9	11	13
	7.19	1.75	8	0	25
	8.99	2.19	15	53	14
	10.78	2.67	6	100	D

Table 27. DD Data: Stimulants in (+)-MBDB-trained rats.

DRUG	DOSE μM/kg	 mg/kg	N	%D	%SDL
(+)-AMP	2.17	0.4	10	20	38
	3.26	0.6	10	20	50
	4.35	0.8	15	47	25
	5.43	1	14	43	38
	6.52	1.2	7	71	0
	10.86	2	15	73	25
MA	0.67	0.13	12	33	13
	1.35	0.25	12	33	38
	2.03	0.38	14	57	17
	2.7	0.5	5	100	D
COCAINE	7.36	2.5	12	25	33
	14.71	5	9	11	50
	29.42	10	9	11	63
	36.78	12.5	5	100	D

Table 28. DD Data: Time delay tests of *S*-(+)-MDA and MDMA in (+)-amphetamine-trained rats.

DRUG	DOSE µM/kg	DOSE mg/kg	Time[a]	N	%D	%SDL
(+)-MDA	3.82	0.823	30	13	38	25
			45	5	20	25
			60	11	36	0
			90	2	0	0
	4.64	1.0	60	10	30	0
			90	11	27	0
	5.80	1.25	60	11	27	13
			90	8	0	25
MDMA	7.63	1.75	30	8	0	25
			45	9	67	0
			60	11	55	0
			90	8	13	14
	9.79	2.25	60	4	100	D
			90	8	75	50

[a] Time = minutes between the time of injection and the beginning of the test session.

Table 29. DD data: 5,6-MDAI behavioral profile.

TRAINING DRUG	5,6-MDAI DOSE µM/kg	mg/kg	N	%D	%SDL
MDMA	1.17	0.25	8	0	13
	2.34	0.5	10	20	50
	4.68	1	8	0	63
	9.37	2	10	10	100
(+)-MBDB	1.17	0.25	8	0	25
	2.34	0.5	8	0	63
	4.68	1	9	11	75
	9.37	2	10	20	100
(+)-AMP	1.17	0.25	8	0	0
	2.34	0.5	9	11	0
	4.68	1	12	33	0
	9.37	2	5	100	D
LSD	0.56	0.13	11	27	13
	1.17	0.25	16	50	50
	2.34	0.5	10	20	38
	4.68	1	15	47	25
	9.37	2	6	83	D

VITA

VITA

Robert Oberlender was born on July 25, 1956 to Martin and Betty Oberlender in Philadelphia, Pennsylvania. After graduating from high school in 1974, he attended Wilkes College in Wilkes-Barre, Pennsylvania, where he studied biology for two years. He then transferred to Temple University in Philadelphia where he became a member of the Rho Chi Pharmacy Honorary Society and received a B.S. in Pharmacy in 1979. With the completion of his undergraduate training, Robert worked for two years in hospital pharmacy, enjoying the fresh air and mountains of Northeastern Pennsylvania.

In August, 1981, he began his graduate school studies at Purdue University, Department of Medicinal Chemistry and Pharmacognosy. Under the direction of his major professor, Dr. David E. Nichols, Robert completed his coursework and began research in synthetic medicinal chemistry and behavioral pharmacology while living in the pleasant comfort of the Waldron House. He was supported for three years by a United States Public Health Service National Research Service Award through a Chemical-Pharmacology Training Grant from the National Institute of General Medical Sciences. During his graduate school training, Robert was elected as a member of the Phi Lambda Upsilon Chemistry Honorary Society and the Sigma Xi Scientific Honorary Society. He graduated with a Ph.D. in May 1989 and began his post-doctoral training in Pharmacology with Dr. Nichols.